INHERITANCE OF POLYDACTYLISM IN THE FOWL[1]

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POLYDACTYLISM, where studied in vertebrates, has usually been shown to be a heritable characteristic, although in some instances the mode of inheritance has been somewhat involved.

In the case of the fowl, the usual number of toes is four although five toes constitute a breed characteristic in some instances. The four-toed condition of the fowl is the result of the loss of the fifth digit from the typical pentadactyl foot of higher vertebrates. It has been shown by various workers (KUAFMANN-WOLF 1908; HARMAN and ALSOP 1938) that the additional toe in five-toed breeds does not constitute a restoration of the missing fifth digit but is the result of the development of a new toe on the opposite side of the foot. The work of BOND (1920, 1926) and PUNNETT and PEASE (1929) has indicated that the five-toed condition behaves genetically as a dominant to four toes. Certain irregularities in the expressions of the character including heterodactyly and the occasional lack of penetrance have been noted. A considerable body of data on polydactylism has been accumulated by the writer in connection with other genetic studies on the fowl. Polydactyly, as here studied, cannot be referred to any particular breed, since it was present in crossbred stocks used in various genetic studies, but the original stocks were Silkie Bantam and Houdan. It was the purpose of this study to obtain further information on genetics of polydactylism in the fowl.

INHERITANCE OF FIVE TOES

Several workers (BOND 1926; PUNNETT and PEASE 1929) have studied the inheritance of the five-toed condition as found in some standard breeds of poultry. The conclusions were that polydactylism of this type behaved as an autosomal dominant, with some of the individuals carrying the gene failing to show extra toes. HUTCHINSON (1931) suggests that the occasional normal individual resulting from a mating involving homozygous dominant polydactylous parents may be due to genetic modifiers influencing dominance. The numbers of birds included in previous studies have been somewhat small, and therefore additional data are presented by the writer. The large numbers recorded for heterozygous polydactyls backcrossed to normals are from linkage tests.

Certain facts are evident from the data in table 1. First the earlier evidence of the dominant behavior of polydactylism in confirmed. It is also noted that the ratios show a deficiency of polydactyls. The greatest deficiency is found where the segregating polydactyls are all heterozygotes (backcrosses to normals). The shortage in these crosses is greater than in the F_2 generation, indicating the possibility that the failure of expression of the character is greater

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TABLE 1

Inheritance of polydactyly (five toes).

	FEMALES		MALES		TOTAL	
	POLY-DACTYLS	NOR-MALS	POLY-DACTYLS	NOR-MALS	POLY-DACTYLS	NOR-MALS
Homozygous polydactyl ♂ × normal ♀	115	5	107	4	222	9
Homozygous polydactyl ♀ × normal ♂	32	0	17	2	49	2
F_2 generation	148	49	143	69	291	118
Backcross of heterozygous polydactyl ♀ to normal ♂	298	359	286	440	584	799
Backcross of heterozygous polydactyl ♂ to normal ♀	818	1247	794	1207	1612	2454
Backcross of heterozygous polydactyl ♀ to homozygous polydactyl ♂	87	4	87	3	174	7
Backcross of heterozygous polydactyl ♂ to homozygous polydactyl ♀	10	1	13	0	23	1

in heterozygotes than in homozygotes. There seems to be a slightly weaker penetrance of the character in males than in females, but the difference is so slight as to be of questionable significance.

The shortage of polydactyls might be due to a number of conditions such as the action of genetic suppressors or inhibitors, to poor penetrance of the factor, or to low viability of birds showing the character. General observations would lend little support to the view that the last mentioned possibility will account for the shortage. As to the existence of genetic inhibitors, the data in table 1 indicate the possible presence of such inhibitors. If the polydactylous stock carried genetic inhibitors, the greater shortage should occur in the F_2 generation or in backcrosses to polydactyls, unless the action of the inhibitor is limited to heterozygous polydactylism. Table 1 shows the greatest deficiency of polydactyls in the backcrosses to normal. Most of the backcrosses of heterozygotes to normals involved purebreds on the normal side of the cross, and these purebreds would ordinarily not be expected to carry inhibitors for polydactylism which they themselves did not carry. The fact that a breed does not carry a factor, however, is no assurance that it does not carry modifying factors or inhibitors for the same. If the deficiency of polydactyls in backcrosses to normals is due to genetic inhibitors, the action must be due to either the normal stocks carrying the same recessive inhibitor found in the polydactyls or to a dominant found in the purebreds. Any dominant inhibitor in the polydactylous stock would tend to suppress polydactylism in the heterozygotes. It remains doubtful, therefore, as to how to account for the observed shortage of polydactyls. The shortage in the offspring of 22 males heterozygous for polydactylism when backcrossed to normals ranged from 0 to 60 percent.

STURKIE (1942) has made a preliminary report on the effect of low temperatures during the early embryonic development upon polydactylism. He reported shortages (normals) of from 18 to 78 percent among heterozygous poly-

dactyls. This is good evidence that the expression (penetrance) of polydactylism is dependent upon environmental factors during chick development. Factors such as this may be the cause of the shortage of polydactyls shown in table 1. That this can be entirely due to temperature fluctuations seems questionable, since the individuals reported here were produced over a period of

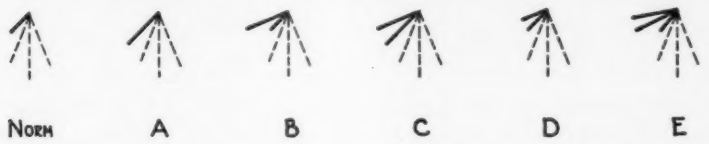

FIG. 1. Diagrammatic representation of the arbitrary classification of polydactylism used in this study. Type A, polyphalangy; type B, the usual type of polydactylism in five-toed breeds; type C, most common type of duplicate; type D, common in the usual type of polydactylism; type E, another common type in duplicate. The diagram listed as normal represents the non-polydactylous four-toed foot.

years under what were believed to be optimum incubation temperatures. In one hatching season two heterozygous polydactylous males were backcrossed to normal females, whose eggs were set weekly and hatched in the same incubator over a period of several weeks with the following results: male 1289, 34 normals to 169 polydactyls and male 1290, 131 normals to 121 polydactyls. It would appear that these divergent ratios could not be due to temperature fluctuations alone. It seems probable that the variation in the expression of polydactyly is due to both genetic and environmental factors.

DUPLICATE

The writer (1941) described a new type of polydactylism which varied somewhat from the usual expression. It frequently showed conspicuous abnormality of the wing as well as the foot digits. BARFURTH (1911) has described minor wing deformities in usual type of polydactylism. This new factor showed other differences in the expression of polydactylism of the feet. Duplicate behaved as a dominant toward normal and its relation to usual five-toed polydactylism will be discussed later.

POLYPHALANGY

In studies of the usual type of polydactyly, another variant has been observed, which has been called polyphalangy. It appears only in polydactylous stocks and constitutes a reversion to the four-toed condition but is characterized by the inner toe (No. 1) having an extra phalanx and being relatively long (fig. 2A). The usual polydactylism (fig. 2B) is the result of what seems to be the addition of a long toe at the inside base of the No. 1 toe. This additional toe has one more phalanx than does the No. 1 toe, and polyphalangy has the appearance of a five-toed polydactyl with the No. 1 toe missing. Since polyphalangy appears only in polydactylous stock, it seems to be a modified

polydactyl. The evidence early indicated that this variant of polydactylism was inherited, but several generations of selection failed to produce a stock of polydactylous birds breeding true for polyphalangy.

The mode of inheritance of the modified polydactylism referred to as polyphalangy appears to be complicated. Data from matings of polyphalangy are given in table 2. That this character is inherited is well demonstrated by comparing the F_2 generation results of the polyphalangy and the polydactyly matings. In the polyphalangy F_2 generation where both parents were polydactyls of the polyphalangeal type (matings 12 to 15) the ratio of polydactyls to polyphalangyls was 241 to 280 while in polydactyly F_2 where both parents were polydactyls not showing the polyphalangeal type (mating 17) the ratio

TABLE 2

Inheritance of Polyphalangy.

	FEMALES			MALES			TOTAL	
	NORMAL	POLYDACTYL	POLYPHALANGYL	NORMAL	POLYDACTYL	POLYPHALANGYL	POLYDACTYL	POLYPHALANGYL
Heterozygous polyphalangy ♂ by normal ♀								
Mating 1—Male 1526	19	5	7	20	1	3	6	10
Mating 2—Male 1528	2	12	9	2	9	1	21	10
Mating 3—Male 1546	4	36	9	1	33	9	69	18
Mating 4—Male 1562	47	13	26	43	13	20	26	46
Mating 5—Male 1579	14	12	0	10	15	0	27	0
Mating 6—Male 1606	17	15	1	23	18	1	33	2
Mating 7—Male 1606	17	13	10	27	17	5	30	15
Mating 8—Male 1610	51	23	4	52	20	1	43	5
Total	171	129	66	178	126	40	255	106
Normal ♂ by heterozygous polyphalangy ♀								
Mating 9—Male 1592	88	36	22	85	45	14	81	36
Mating 10—Male 1634	22	18	8	17	11	7	29	15
Mating 11—Male 1555	17	32	4	29	36	0	68	4
Total	127	86	34	131	92	21	178	55
Polyphalangy F_2								
Mating 12—Male 1546	4	53	34	3	67	20	120	54
Mating 13—Male 1606	12	14	30	16	19	30	33	60
Mating 14—Male 1610	22	16	34	30	15	28	31	62
Mating 15—Male 1637	22	27	51	25	30	53	57	104
Total	60	110	149	74	131	131	241	280
Polydactylous matings								
Mating 16—Polydactyl by normal	331	266	11	296	278	15	544	26
Mating 17—Polydactyly F_2	10	46	4	21	61	2	107	6

was 107 polydactyls to six polyphalangyls. Since polyphalangy appears to be a modification of polydactyly, the normals in the table may be disregarded. In some matings one of the parents may have been homozygous for the factor for the usual type of polydactyly, but the data of significance here are those showing the ratio of a polyphalangyls to non-polyphalangyls among the polydactyls. It is true that only four-toed polydactyls having a long inside toe (fig. 1, Type A) were classed as polyphalangeals in table 2. Later analyses in this study seem to show that types C and E (fig. 1) may also carry the polyphalangeal factor. However, the numbers of these two types were not large and if accounted for would not greatly change the ratios in table 2.

Reciprocal F_1 matings between normal and polyphalangy show no evidence of sex-linked factors being involved. The F_1 generation shows wide variability as to the incidence of polyphalangy ranging from zero to 64 percent of the polydactyls exhibiting the modification. The variability might be due either to differences in the genetic constitution of the parents or to the influence of some environmental factor. The two matings (matings 6 and 7) listed for male 1606 involve two different groups of normal female mates. The term normal as used in table 2 indicates the absence of any type of polydactyly. The White Leghorn female mates in mating 7 produced a much larger proportion of the polyphalangeal type than did the Rhode Island Reds in mating 6. The same Rhode Island Red females were mated with males 1579 and 1610 in matings 6 and 8, and in each case gave a low incidence of polyphalangy in the F_1 generations. These results might be taken to indicate genetic differences among the purebred non-polydactylous stocks as to the presence of the modifier converting polydactyly into polyphalangy. In view of the evidence, however, that the expression of other forms of polydactyly in the fowl is influenced by environmental factors, it is possible that such factors are also acting here.

The F_2 generation ratio is also probably influenced by the factors causing variability in the F_1 generation. It would seem then that polyphalangy is a factor which behaves as an autosomal dominant with some unknown factors causing variability in dominance.

EXPRESSION OF POLYDACTYLY IN THE DIFFERENT GENETIC STOCKS

In studies of polydactyly it was evident that most of the variants fell into five major types which are illustrated diagramatically in figure 1. Photographic examples of the types are found in figure 2. In the polydactylous fowl the digits 2, 3, and 4 seem to be unaffected, and in some types even digit 1 remains normal. The classification in figure 1 is based upon the relative lengths of extra toes in comparison to digit 1. In the normal four-toed foot the No. 1 digit is relatively short, possessing one phalanx and the nail. The ordinary type of polydactyly (type B, fig. 1) seems to possess an unaffected No. 1 digit with an extra digit added to the outside of this digit (on inside of foot). The extra digit has an additional phalanx making it longer than the No. 1. Polyphalangy (type A) has the appearance of type B with the No. 1 digit missing. Type C has two long extra digits and might be considered to be type A with the long

inside toe split. Type D possesses an extra toe which is short. Type E has six toes with the appearance of being a combination of the normal and type C. Variants of polydactyly occur which do not fit the foregoing classifications, but in general the suggested groupings are quite satisfactory for a large majority of the expressions.

Table 3 presents data showing the incidence of these variants of polydactyly as found in the polydactylous (usual) matings, polyphalangy matings, and duplicate matings. Heterodactyls have not been included in this table

TABLE 3

Incidence of types of polydactyly in different stocks.

TYPE OF OFFSPRING	STOCKS MATED					
	USUAL POLYDACTYLY		POLYPHALANGY		DUPLICATE	
	NO.	PERCENTAGE	NO.	PERCENTAGE	NO.	PERCENTAGE
A	13	2	222	39	25	3
B	527	85	248	44	152	16
C	3	0	51	9	337	36
D	70	11	42	7	24	3
E	4	1	6	1	397	42
Total	617		569		935	

because of difficulty of classification. Polyphalangy differs from the usual five-toed type in the distribution of its variants only by showing an increase of types A and C. Agreement in the other classes might be expected, since polyphalangy appears to be a modification of the five-toed type. Duplicate, which differs considerably from the usual polydactyly, shows a high incidence of the six-toed, type E which is rarely found in the other two stocks. It also shows a large proportion of type C. The differences found in the proportion of types of polydactyls appearing in the stocks is evidence of genetic diversity. Similarity of types as found in the three different stocks makes it impossible to determine origin of some individuals without reference to their pedigree, but breeding tests reveal differences. The type E (fig. 1) is found commonly in duplicate, but virtually never in polydactylous and seldom in polyphalangeal stocks. An example of the difference in breeding behavior of types E from duplicate and polyphalangeal stocks is found in table 4. The data in table 4 were presented for another purpose, but a comparison of data obtained under normal temperature conditions affords a striking contrast of the expression of polydactylism among offspring of males identical in appearance but originating in duplicate and polyphalangeal stocks. The four males used for matings reported in table 4 were virtually indistinguishable but those from the duplicate and polyphalangeal stocks differ widely with respect to the relative incidence of the various types of polydactyly.

Following the suggestion of STURKIE (1942) that low incubation temperatures influenced the expression of polydactyly, an experiment was planned to test the effect of this factor on the types of polydactylism obtained. STURKIE considered primarily the tendency of subnormal incubation temperatures to suppress polydactylism. Since there was reported in the present study considerable variation in the types of polydactyly obtained in the different stocks, it is of interest to determine whether incubation temperatures were influencing factors. DR. STURKIE kindly advised the writer as to what temperature changes

TABLE 4

Effect of lowered incubation temperatures on expression of polydactyly.

MALE NO.	TREATMENT	NO. OFF-SPRING	PERCENTAGE OFFSPRING DESCRIBED AS—						
			NOR-MAL	TYPE A	TYPE B	TYPE C	TYPE D	TYPE E	HETERO-DACTYL
Duplicate	Normal	302	42		1	9		39	9
2407	Cold	33	46	3	12	18		9	12
Duplicate	Normal	442	52	2	5	16		8	17
5372	Cold	52	48	15	6	12		4	15
Polyphalangy	Normal	127	1	14	32	2	12	2	39
4987	Cold	69	12	32	17	1	10		28
Polyphalangy	Normal	108	1	47	10	7	3	3	29
1729	Cold	17	11	77	6				6

he was finding most effective in suppressing polydactylism. The procedure here followed was to carry the eggs at normal incubation temperatures (100°F) for the first 48 hours. Then the eggs were placed in a large refrigerator for 16 hours where temperatures varied from 37 to 40°F. For the next 72 hours the incubation temperature was 93°F, following which normal incubation temperatures were maintained for the remaining incubation period. The embryonic mortality was somewhat high, but all late stage embryos and hatched chicks were classified as to the type of polydactyly. Those individuals listed in table 4 as being subjected to normal incubation temperatures were produced by the same parent stock before and after the period of low incubation temperature conditions. Males 2407 and 5372 were type E duplicates, and males 4987 and 1729 were type E polyphalangyls. The first three named males were mated with White Leghorn females, and male 1729 was mated with polyphalangeal females. STURKIE (1942) found polydactyly to be suppressed in Houdans (mostly of type B) by the cold treatment here used. The results in table 4 indicate only a slight increase in the number of normals (no type of polydactyly) due to cold treatment in the polyphalangyls and virtually no influence on the duplicates. In the matings of polyphalangyls there was a definite increase in the percentage of type A (14 to 32 percent and 47 to 77

percent) and a corresponding reduction in the percentage of type B. In the duplicate matings cold treatment resulted in reductions in the incidence of the type E with increases in the types A and B. It would seem, therefore, that the incidence of the various types of polydactyly in the different stock may be the result of environmental as well as inherent factors. Extremely low temperatures such as here provided would seldom be encountered in the incubation period, but it is of interest to record that the expression of polydactyly is influenced by environmental factors. The writer failed to obtain the degree of suppression of polydactyly such as reported by STURKIE, but this may be due to differences in the stocks utilized in the test.

Because of the limited numbers and irregular distribution of data in table 4, the data are not very satisfactory for statistical analysis. The χ^2 test is probably the best one for material of this type. This test[2] showed statistically significant differences in expression of the polydactyly due to subjecting eggs to cold treatment during the early incubation period. In all the males, except the last one listed, the differences are statistically significant, and for the last one the numbers of treated chicks are small. The results of the test are as follows:

Male 2407 $-\chi^2 = 31.15$, 3 degrees of freedom, P less than .001
Male 5372 $-\chi^2 = 26.05$, 4 degrees of freedom, P less than .001
Male 4987 $-\chi^2 = 20.65$, 5 degrees of freedom, P = .001
Male 1729 $-\chi^2 = 8.75$, 5 degrees of freedom, P = .12

HETERODACTYLY

The phenomenon of heterodactyly in toe numbers has been observed by earlier workers (BOND 1920, 1926; PUNNETT and PEASE 1929). It has been noted that in heterodactylous birds the extra digit more often appears on the left foot. Although other types of heterodactyly occur in polydactyls, the usual reference to heterodactyly in the fowl has been applied to individuals possessing one normal and one polydactylous foot.

In table 5 are shown the types of heterodactyls observed in both the usual polydactylous stocks and in stocks of duplicates. The table is arranged to record the type of polydactyly found in each foot and brings out a number of interesting facts. The homodactyls found in the same matings are not here recorded. Considering first the data for the polydactyls (usual form), it is seen that we have confirmation for the findings of earlier workers that in polydactylous birds if one foot is normal (four-toed), it is more likely to be the right foot. Of the 177 individuals with one normal foot, only 35 showed polydactyly on the right foot, while 142 had the extra toe on the left foot. In the foregoing statement no consideration was given to the type of polydactyly found on the polydactylous foot. No statistical treatment is here necessary to prove that when polydactyly occurs on one foot only, the left foot is more usually affected. It is also evident that the incidence of the type D (fig. 1) polydactyly is high among heterodactyls with one normal foot. Of the 177 heterodactyls

[2] The statistical treatments given in this paper were made by DR. H. C. FRYER, statistician of the KANSAS AGRICULTURAL EXPERIMENT STATION.

of this type, 98 had the type-D polydactyly. Among the homodactyls reported in table 3 only 70 of type D were found in a total of 617. The high incidence of type D among heterodactyls having one normal foot may be taken to indicate that the factors which cause the development of a normal foot also suppress polydactylism in the other foot so that the expression is not normal. Thus type D may be considered as an aberrant or partially suppressed type B.

TABLE 5

Types of heterodactyly.

CONDITION OF RIGHT FOOT	CONDITION OF LEFT FOOT						
	NORMAL	TYPE A	TYPE B	TYPE C	TYPE D	TYPE E	TOTAL
Polydactylous stocks:							
Normal		11	59		72		142
Type A	1		49	20	3	1	74
Type B	8	76		3	25	11	123
Type C		4	3				7
Type D	26	17	57				100
Type E			5	2			7
Total	35	108	173	25	100	12	453
Duplicate stocks:							
Normal		4	11		33	3	51
Type A			19	7	4	11	41
Type B	3	19		19	6	75	122
Type C		4	11		1	83	99
Type D	12	1	25			5	43
Type E	1	3	31	112	1		148
Total	16	31	97	138	45	177	594

The tendency of polydactylism to be more readily suppressed on the right foot is also seen in the portion of table 5 presenting data on duplicate stocks. Of those having one normal foot, 16 showed normalcy on the left foot and 51 on the right foot, which would indicate that when polydactylism is expressed on one foot only, it occurs more frequently on the left foot. Of the duplicate heterodactyls possessing one normal foot, four were of type A, 14 of type B, none of type C, 45 of type D, and four of type E. The proportion of the various types of polydactyly among heterodactyls is very different from those found in table 3 for duplicate homodactyls. In table 3 the types C and E were far more numerous than any others, while they are rare among the heterodactyls. These results may be taken to indicate that the factor which produces one normal foot in a duplicate individual also tends to cause an abnormality in the expression of duplicate on the other foot. The existing combination of the various types of polydactyly in heterodactyls as shown in table 5 should throw further light on their relationships which are discussed later.

INHERITANCE OF VARIANTS OF POLYDACTYLISM

In table 3 are shown the various expressions of polydactylisms in the different stocks. It is seen that the proportion of the various types differs in the three stocks, thus indicating genetic differences among them. Convincing evidence has been presented showing that the polyphalangeal type of polydactyly is due to genetic modifiers. The only other variant occurring frequently in ordinary polydactylous stock is the type D which is somewhat variable in its expression, with the extra toe ranging in length from an atrophied single

TABLE 6

Types of duplicate offspring obtained from different parental types when mated with normals. (Normal and heterodactylous offspring not included.)

TYPE OF OFFSPRING	PARENT OF THE TYPE—					
	B		C		E	
	NO.	PERCENTAGE	NO.	PERCENTAGE	NO.	PERCENTAGE
A	2	3	2	2	4	3
B	32	53	25	28	35	29
C	8	13	43	48	31	26
D	12	20	4	4	2	2
E	6	10	16	18	49	40

phalanx to one of the same approximate length as the No. 1 toe. There were only a few matings involving the type D parents, and these produced a shortage of polydactylous offspring and a high percentage of heterodactyls. This might be taken as evidence for a genetic suppressor which either completely inhibits polydactylism or causes an abnormal type of polydactylism such as the type D. It was earlier found that these two conditions were frequently associated in heterodactyls. However, the data are not adequate to demonstrate existence of such a modifier.

From the data on duplicate stock matings (table 6) there is evidence for genetic factors influencing the type of duplicates obtained. The three most common types of duplicates—B, C, and E (see table 3)—were each mated with normals. Although all five types of duplicates were obtained from each of the parent types, there was a definite tendency for each to produce a high percentage of offspring of its own kind. To obtain such results in matings with normals would mean that any genetic modifiers differentiating duplicate into the three types must be at least partially dominant. The fact that the B and D types of duplicate are identical with the more common expression of the ordinary type of polydactyly might be interpreted as evidence for contamination of the duplicate stock by the polydactylous stock. Such a possibility was early recognized in view of the similarity of expressions, and it is believed that the precautions taken prevented such being the case.

The χ^2 test showed the association between parent and offspring types of polydactyly to be statistically highly significant. The χ^2 value of 63.57 with eight degrees of freedom gave a P much less than .001.

ANATOMY OF POLYDACTYLOUS FEET

In the classification of the variants found in the three stocks of polydactyly—usual type, duplicate and polyphalangy—the individuals fell fairly definitely in the arbitrarily established classes (fig. 1). A few questionable ones were included in type D, since these appeared to be modified type B in which the extra digit failed to develop completely. In such cases the extra toe might vary from a mere suggestion of a nail to a toe approximately as long as the first digit. The data presented earlier seem to indicate that the type D is the expression of the usual type of polydactyly (type B) under the influence of an inhibitor. The careful studies of HARMAN and ALSOP (1938) and HARMAN and NELSON (1941) on the anatomy of polydactylous feet through the use of alizarin preparations showed considerable variation in the number of phalanges and metatarsals. There was evidence of fusion or perhaps failure in separation of the phalanges. Thus the relative length of the digits is not always an accurate measure of the number of bones they carry.

The survey of the various types of polydactyls observed in this study suggests the action of three rather distinct developmental procedures. These steps are not always perfectly executed, thus giving a rather wide range of types of polydactylism. The three steps are: first, the addition of a longer digit outside of the halux or number one digit; second, the splitting of the added digit; and third, the loss of the normal halux or number one digit. It is true that other explanations might be found for the observed types of polydactyls, but the following facts seem to lend support to the hypothesis. First the type B (fig. 1) polydactyl, which is the usual expression found in five-toed breeds, seems to be the result of an addition rather than a splitting of the halux of a normal (four-toed) foot. In the large number of polydactyls observed in this study, there was virtually no evidence of splitting of the halux, although the phenomenon was of common occurrence in the extra digit. Furthermore the studies of HARMAN and NELSON (1941) revealed the fact that the halux and the extra digit usually had separate metatarsals. The fact that the extra toe has one more phalanx than the halux is further evidence that it is not being split off from the halux.

The view that types C and E (fig. 1) are the result of splitting of the extra digit is sustained by the large number of variants of these groups. They show evidence of splitting originating distally and varying from merely a double nail to two distinct toes of equal length. HARMAN and NELSON (1941) found the split toes frequently to have one or more phalanges in common and usually to possess a single metatarsal.

Evidence that types A and C are the result of the loss of the normal halux from a foot carrying one extra digit, split in type C and unsplit in type A, is found in variants of these two types. Frequently the types A and C showed a

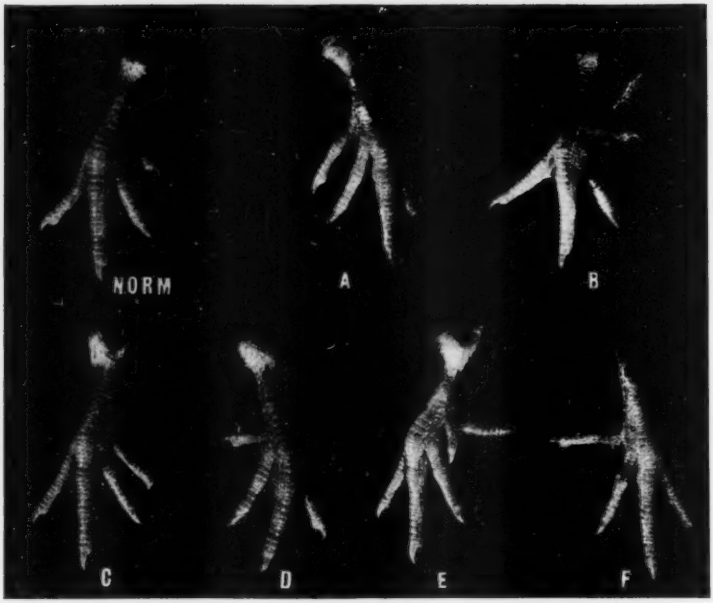

FIG. 2. Illustrating the normal four-toed foot, the five types of polydactylism and (F) an expression of polyphalangy showing a vestige of the missing halux.

vestige (see fig. 2, type F) of the halux in the positions where the digit occurs in types B and E. The vestige of the halux may carry a nail or show only as a thickening at the inside base of the extra toe. If the hypothesis of the loss of the halux is accepted, types A and C may be interpreted as being the result of this action in types B and E, respectively.

Thus far all observed types have been accounted for except type D. As mentioned before, those variants placed in this class are somewhat divergent. It is proposed that those classed as type D are imperfectly expressed type B polydactyls. Support for this view is found in the fact that type D was most commonly observed in stocks carrying the type B polydactylism (see table 3). Furthermore, in table 5 it is seen that in heterodactylous individuals (complete suppression of polydactyly in one foot) there is a high incidence of type D polydactyls. This seems to suggest that type D is an imperfectly developed type B and would thus account for the wide variation in those polydactyls of the type D group.

The data on heterodactyls in table 5 also lend support to the earlier proposal that type A is a modified B, and C a modified E. It is seen that in the heterodactylous foot, types A and B and types C and E are most commonly associated, indicating that developmentally they are related. Likewise the data in

table 3 on incidence of the various types in the different stocks show association of the above mentioned classes of polydactyly.

Inasmuch as it has been shown that some, and perhaps several others of the variants of polydactylism have genetic bases, it is somewhat difficult to see how they are associated developmentally. The distribution of the different types suggests the interaction of the addition of a digit, the splitting of the added one, and finally the suppression of the number one digit of the normal foot. In the usual polydactylous breeds, the development of the extra toe seems to be the only change. In the polyphalangeal stock, the usual type of polydactyly is further modified by the suppression of the normal halux. Polyphalangy also occasionally shows splitting of the additional digit. In the case of the duplicate stock, most individuals show the splitting of the extra digit, and suppression of the normal halux is common.

GENETIC RELATIONSHIPS OF DUPLICATE

Although duplicate has many characteristics in common with the polydactyly found as a standard trait in several breeds of poultry, it also differs sufficiently to demonstrate an independent genetic origin. An earlier publication by the writer (1941) contained the suggestion that duplicate and polydactyly constitute a multiple allelic series since the segregation ratios from crossing the two kinds of polydactylism favored this interpretation. Fortunately the recent studies of HUTT and MUELLER (1943) supplied information which made possible a crucial test of theory of the existence of multiple allelic series for polydactylism. HUTT and MUELLER found that ordinary polydactylism belonged to the same linkage group as did duplex comb and multiple spurs. If duplicate is a mutation of the same gene as is polydactyly, then the former should show linkage relations with duplex comb and multiple spurs similar to those of polydactyly. A mating was set up to test the linkage of duplicate with multiple spurs (repulsion series), since data were already at hand testing linkage of duplicate with duplex comb. The test of two males heterozygous for duplicate and multiple spurs gave the following linkage results: duplicate, non-multiple spurs 187 and 119; non-duplicate, multiple spur, 149 and 104; duplicate, multiple spurs 46 and 67; nonduplicate, non-multiple spurs 89 and 37 where the first two groups constitute the parental combinations and the latter two the new combinations. The combined results of the two tests gave a crossing over percentage of 30. A total of 191 individuals gave a crossover percentage of 43 percent between duplicate and duplex comb.

In table 7 is given a comparison of the linkage relations of duplicate and polydactyly with duplex comb and multiple spur. The table carries data of both the writer and HUTT and MUELLER (1943). It is seen that duplicate gives crossing over data with duplex and multiple spur similar to that obtained from polydactyly with the same characters. The crossover percentages for duplex with polydactyly and with duplicate were 46 and 43, respectively, and for multiple spurs were 34 and 30. Some of the difference in percentage crossing over is probably due to variation in the degree of expression of polydactyly

TABLE 7

Comparison of linkage relations of polydactyly and of duplicate.

CHARACTERS TESTED	PERCENTAGE CROSSING OVER REPORTED BY	
	HUTT AND MUELLER	WARREN
Polydactyly—duplex	42	46
Duplicate—duplex	—	43
Polydactyly—multiple spur	34	—
Duplicate—multiple spur	—	30
Duplex—multiple spur	28	28

noted by numerous workers and its effect on linkage data reported by HUTT and MUELLER (1943). The writer's data on the linkage relation of polydactyly and duplex involved 1079 individuals; however, the elimination of matings showing striking shortages of polydactyls failed to significantly change the crossover value of 46. In the case of matings involving duplicate and multiple spurs there was probably some error due to the duplicate character so extremely deforming the leg that multiple spurs were not easily identified. The shortage of polydactyls in the duplicate stock was not so great as that observed in the usual form of polydactylism.

The linkage data in table 7 fairly definitely substantiate the earlier proposal that duplicate and the usual type of polydactyly are probably mutations at the same locus, thus constituting a multiple allelic series.

SUMMARY

Three strains of polydactylous fowl, the usual five-toed type, a polydactylous stock selected for polyphalangy, and the duplicate type of polydactylism, were investigated. Each of the three stocks showed a distinctive incidence of the various expressions of polydactylism.

An analysis of data on heterodactyly indicated that some of the variations in expression of polydactylism were the result of partial suppression of this character. The suppression appeared to be due to both environmental and inherent factors. Low temperatures during the early incubation period influenced the expression of polydactylism.

It is proposed that expression of polydactylism is accomplished by varying combinations of three processes—the addition of a digit beside the No. 1 digit; the splitting of the added digit; and the loss of the normal No. 1 digit.

Polyphalangy appeared to be due to an incompletely dominant modifier of the usual five-toed type of polydactylism. That the usual type of polydactylism and duplicate are each dominant mutations at the same locus is indicated by segregation ratios and by linkage relations. There is also evidence that the expression of polydactylism in three stocks studied is influenced by minor genetic factors.

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CYTOGENETIC STUDIES WITH POLYPLOID SPECIES OF WHEAT. II. ADDITIONAL CHROMOSOMAL ABERRATIONS IN TRITICUM VULGARE[1]

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INTRODUCTION

MONOSOMES are very useful in the genetic analysis of a species, as CLAUSEN (1941a) has pointed out, since they greatly facilitate the locating of genes on the chromosomes. The loss of an entire chromosome from a strictly diploid organism, however, is too deleterious to be tolerated; and thus the method of monosomic analysis is possible only in polyploid organisms, where the added chromosomes tend to counteract the effects of chromosome losses.

Of still greater value for genetic analysis where they can be obtained are nullisomes, which are deficiencies for both members of a pair of chromosomes. Only in the higher polyploids, however, such as the allohexaploid *Triticum vulgare* (n = 21), common wheat, are nullisomics viable.

During the past several years, numerous nullisomes, tetrasomes, and other aberrations have been accumulated in *T. vulgare*. An account of the origin of part of this material was given in the previous paper of this series (SEARS 1939), and a brief description of seven nullisomics was published in 1941. The present report aims to bring the account up to date. More detailed presentation of some of the data will be made in subsequent papers.

MATERIALS AND METHODS

All of the aberrations have occurred in the wheat variety Chinese Spring. As described in a previous publication (SEARS 1939), 16 monosomes and five trisomes were found in the 11 aberrant offspring of a monoploid pollinated by a diploid. Through selfing of plants possessing these aberrations, the corresponding nullisomes and tetrasomes have been obtained. One of these nullisomes, which renders plants partially asynaptic without unduly depressing their fertility, has provided a ready source of additional monosomes and trisomes. A few monosomes and nullisomes were kindly supplied by DR. J. G. O'MARA, from certain of his cultures of wheat-rye derivatives.

Part-chromosome aberrations, consisting of telocentric chromosomes and isochromosomes, have occurred frequently. They have appeared mainly in offspring of monosomic plants, presumably following misdivision of univalents.

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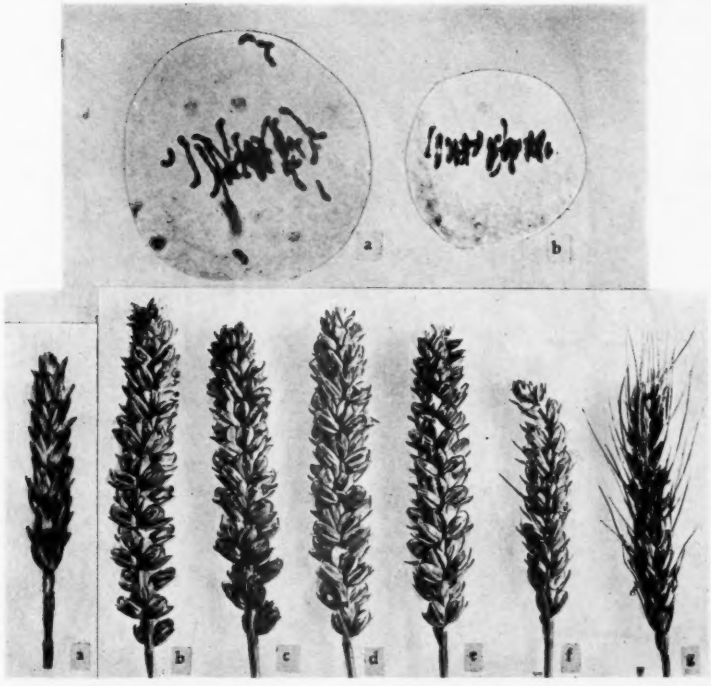

FIG. 1 (above). MI in microsporocytes from (a) nullisomic-III and (b) normal plants. The former cell shows a reduced frequency of chiasmata and six univalent chromosomes. ×547 and 456, respectively.

FIG. 2 (below). Spikes showing ability of tetrasome II to compensate for nullisome XX. (a) Nulli-XX, (b) mono-XX, (c) mono-XX, tri-II, (d) normal, (e) nulli-XX, tetra-II, (f) tri-II, (g) tetra-II. All ×0.625

Cytological observations were made from microsporocytes smeared in aceto-carmine after fixation of whole spikes for about two days in Carnoy's solution. Simple determinations, such as the number of monosomes present, were frequently made with a high-dry objective from preparations with no cover slip. Most other observations were from freshly prepared slides, although some were from permanent preparations. Most of these permanent slides were made by the tertiary-butyl-alcohol method outlined in a previous publication (SEARS 1944).

MONOSOMES AND NULLISOMES

Review of literature

Although monosomes have been observed fairly frequently in polyploid species, nullisomic plants have seldom been reported. CLAUSEN (1941a) has isolated at least 20 of the 24 possible monosomic types in *Nicotiana Tabacum*, but thus far he (1941b) has obtained no nullisomics. In hexaploid *Avena sativa*, HUSKINS (1927) found that certain homozygous fatuoids (resembling *A. fatua*) have only 20 pairs of chromosomes, and PHILP (1935, 1938) isolated two additional nullisomics.

In the hexaploid wheats (Triticum) nullisomics have been reported by a number of investigators, but many of these deficiencies have evidently involved the same chromosome, the "C" or speltoid chromosome. Speltoidy (so called because of the resemblance to *T. spelta*) has been shown by WINGE (1924), HUSKINS (1928), PHILIPTSCHENKO (1929), HÅKANSSON (1930), BYNOV (1938a), and MATSUMURA (1939) frequently to be due to the effects of a particular monosome or its corresponding nullisome. Nullisomic dwarfs or semi-dwarfs have been reported by KIHARA (1924) among derivatives of *T. polonicum*×*T. spelta*, by THOMPSON (1928) following a varietal cross in *T. vulgare*, and by UCHIKAWA (1938). LOVE (1940) found several nullisomic plants and even nullisomic lines in 50 strains (F_5 to F_7) selected for agronomic characters from hybrids of *T. vulgare*×*T. durum;* most of these nullisomics were to some extent fertile.

Several dwarfs in wheat have been observed which were not thoroughly investigated cytologically, if at all, and some of these may have been nullisomic types. Such dwarfs were reported by FARRER (1898), RICHARDSON (1913), NEETHLING (1917), CUTLER (1919), THOMPSON (1922), and WALDRON (1924), mostly following varietal hybridization. The occurrence of normal plants among the offspring of certain of these dwarfs may be explained as the result of natural cross-pollination of nullisomics by normal plants. Nullisomics, which are frequently of reduced male fertility, are particularly subject to such crossing. The crossed seeds would have yielded monosomic plants, which would probably have been reasonably normal in appearance. The absence of dwarfs in the progeny of these normal-appearing plants could have been due to too small populations, since nullisomics are rather rare in the progeny of certain monosomics.

Chiefly because the nullisomics which have been reported in hexaploid wheats have occurred for the most part in different varieties, or even in

derivatives from hybrids with tetraploid species, it is impossible to conclude how many different chromosomes have been concerned. It seems probable, however, that the number is comparatively low. Most of the deficiencies (except those reported by LOVE) have been discovered first in monosomic condition, through morphological peculiarities of the monosomic plants; and the present investigation indicates that there are few monosomics in *T. vulgare* which differ appreciably from normal.

Description of nullisomic plants

Seventeen of the possible 21 different nullisomes in *Triticum vulgare* have now been obtained. These have been designated I to XI and XV to XX, respectively, according to whether or not the missing chromosome is homologous to any of the 14 chromosomes in the haploid complement of *T. durum*. Within these two groups the chromosomes have been numbered according to the order in which the nullisomic types were found. Nullisomics I to V, XV, and XVI have already been briefly described (SEARS 1941).

Only the most striking characteristics of the nullisomic plants will be noted here, since a complete description is contemplated when as many as possible of the remaining four nullisomics have been obtained.

Nullisomic I. Spikes less dense than normal. Glumes darker-colored and somewhat stiffer. Fertility 50 percent or more in some early spikes but usually zero in later spikes.

Nullisomic II. Tillering much reduced. Maturity delayed. Culms short and thick. Leaves broad. Spikes short and thick, frequently with one or more reduplicated spikelets. Female sterile. Completely awnless, whereas normal plants have "hooded" (reduced and recurved) tip awns.

Nullisomic III. Culms short. Leaves narrow. Spikes short and compact. Two or more univalents usually present at meiotic metaphase. Fertility fair, with seed set reaching 100 percent in some artificially pollinated spikes.

Nullisomic IV. Narrow culms and leaves. Spikes nearly normal in appearance, but usually male sterile.

Nullisomic V. Culms short and thin. Leaves narrow. Spikes small, with small glumes and indehiscent anthers. Maturity delayed.

Nullisomic VI. Culms short and thin. Leaves narrow. Spikes short and of straggly appearance due to narrow, spreading outer glumes. Fertility low.

Nullisomic VII. Most nearly normal of the 17 nullisomics. Culms slightly shortened, and fertility reduced in upper portion of spike.

Nullisomic VIII. Strongly increased tillering. Culms much reduced in length and diameter. Leaves narrow. Spikes small, with awns straight and somewhat longer than normal. Maturity delayed. Male sterile.

Nullisomic IX. Homozygous speltoid. Culms and leaves narrow. Nonpubescent nodes. Maturity delayed. Spikes lax, narrow. Glumes small and stiff. Awns somewhat increased in length. Male sterile.

Nullisomic X. Culms short and thin. Leaves narrow. Maturity delayed. Spikes short. Outer glumes slender and spreading. Awns distinctly longer than normal. Male sterile.

Nullisomic XI. Fertility reduced. Two or more ovaries (up to four) in occasional florets.

Nullisomic XV. Thin culms and narrow leaves. Spikes short and usually dense. Male sterile, although anthers dehisce.

Nullisomic XVI. Culms short. Leaves narrow. Spikes short, very dense, and frequently bent or curled. Maturity delayed. Fertility very low. Seeds white instead of the normal light red.

Nullisomic XVII. Culms short and thin. Spikes lax and tending to be shortened by abortion of terminal spikelets. Fertility strongly reduced.

Nullisomic XVIII. Extremely fine-leaved and weak in seedling stages, but reasonably vigorous at maturity. Very late. Spikes small and slender. Reduced female fertility. Apparently male sterile, although anthers dehisce.

Nullisomic XIX. Long, lax spikes with a straggly appearance due to outer glumes being narrow and spreading. Fertility reduced.

Nullisomic XX (fig. 2a). Very similar to nulli-II, except slightly more vigorous, less coarse, and slightly female fertile.

To summarize the data on fertility of the 17 nullisomics, none is completely sterile, ten or 11 are fertile to some extent as both male and female, five or six are fertile on the female side only, and one is fertile on the male side only.

The nullisomics show the location of several of the known genes in wheat. A gene for red seeds is shown to be located on chromosome XVI—that is, on the chromosome which is absent in nullisomic XVI. The factors for pubescent nodes, squareheadedness and suppression of speltoidy, already known to be linked together on the speltoid chromosome, prove to be located on the chromosome here designated IX. Data are also supplied concerning the genes affecting awn development. WATKINS and ELLERTON (1940) find Chinese wheat to have the genetic constitution *b1b1 B2B2 HdHd*, where *B2* largely suppresses awn development and *Hd* results in slight further suppression as well as causing the awnlet to be recurved into a hook or "hood." *Hd* is located on chromosome VIII. *B2* evidently lies on chromosome X. The fact that deficiency for chromosome IX, which is known to carry the factor *b1*, results in an increase in awn development suggests that this recessive gene functions in the same manner as its dominant allele but less actively. Whether *b2* may be of this type also cannot be ascertained, since no *b2b2* material is available for comparison with nullisomic X. Two previously unknown factors affecting awns are indicated by the complete awnlessness of nullisomics II and XX. These factors are of opposite type to the other known awn genes, in that they promote awn development rather than inhibit it.

Characteristics of monosomic plants

Under the most favorable conditions of environment, few of the monosomics corresponding to the 17 different nullisomics differ appreciably from normal. Monosomic IX, which represents the heterozygous speltoid condition, shows the typical speltoid effect of thicker, tougher glumes, as well as lax, tapering, non-"squarehead" spikes. Monosomic XVIII is of distinctly later maturity than normal.

Under less favorable environmental circumstances there may be observed some departure from the normal phenotype in the direction of the nullisomic. The amount of this departure seems to depend upon the degree of the difference between the particular nullisomic and the normal. Thus mono-I plants are seldom distinguishable from normal, while mono-II may deviate so far as to be practically awnless and largely female sterile. So far as has been observed, the monosomic exhibits no peculiarity which does not appear to an exaggerated degree in the nullisomic.

The tendency of monosomic plants to resemble their corresponding nullisomics accounts for the fact that certain monosomics among the immediate offspring of the original monoploid were somewhat abnormal (SEARS 1939), for this family was raised to maturity in the greenhouse under rather crowded conditions. In the next generation, which was grown in the field under favorable conditions, certain monosomes were also reported to cause reduced vigor; but only abnormal-appearing plants were examined cytologically in these families. These plants happened to be monosomic, as presumably were most of the plants classed as phenotypically normal. The statement concerning the progeny from plant 2, that "the only monosomic which occurred was dwarfed and sterile," has proved upon re-examination of the cytological preparation obtained from this plant to have been incorrect. The plant was definitely nullisomic (for chromosome II), and its sibs, which were classed as normals on phenotypic grounds, were presumably mostly monosomic. The seed from two sibs has since been grown, and these two plants were both monosomic.

Transmission of monosomes

Since the transmission of monosomes results in normal rather than monosomic or nullisomic offspring, it is less confusing to speak of the transmission of the deficiencies concerned. Thus, it may be said that mono-I plants have deficiency I and that this deficiency is transmitted by means of deficient-I gametes to the mono- and nulli-I offspring.

When monosomic plants are selfed, nullisomic offspring are produced in frequencies ranging from about one to ten percent. In table 1 are presented the data available concerning the frequency of occurrence of each of the 17 nullisomics. The data are less accurate for some nullisomics than for others, because classification of nullisomics was sometimes made without cytological examination, frequently at the seedling stage. This procedure introduced at least two sources of error: (1) The difficulty of separating certain nullisomics from monosomic or normal sibs at seedling stages. This is particularly true of nullisomics I, VII, and XI, and, under certain conditions, of nullisomics II, XVII, XIX, and XX. (2) The resemblance to nullisomics shown by certain plants with a telocentric or iso-monosome. This phenomenon, which is due to the fact that most or all of the factors on a particular chromosome affecting the morphology of the plant may be located on one arm of that chromosome, has been observed for chromosomes II, III, IV, VI, IX, and X. It will be noted that these two sources of error operate in opposite directions, the first tending to decrease the number of nullisomics recorded (since seedlings classified as

TABLE 1

Frequency of nullisomics in progenies from self-pollinated monosomics.

MONOSOME INVOLVED	NUMBER SEEDS PLANTED	NUMBER PLANTS OBTAINED	PERCENTAGE NULLISOMIC
I	403	393	1.8
II	669	613	6.4
III	1027	1009	10.3
IV	977	933	6.3
V	968	937	0.9
VI	160	153	4.6
VII	50	49	4.1
VIII	110	108	2.8
IX	135	134	1.5
X	290	278	1.8
XI	205	199	2.5
XV	1263	1232	6.2
XVI	726	712	5.8
XVII	300	296	3.0
XVIII	503	492	1.0
XIX	305	293	3.1
XX	190	187	3.7

non-nullisomic were discarded), and the second tending to increase the number recorded. On the whole, the data are believed to be reasonably reliable. Particularly trustworthy are the figures for the three nullisomics which occurred least frequently, V (0.9 percent), XVIII (1.0 percent), and IX (1.5 percent). The value for the most frequently occurring nullisomic, III (10.3 percent), is also probably fairly accurate.

The observed frequencies of nullisomics are of the same order as those previously reported for nullisomics in wheat (HUSKINS 1928; NISHIYAMA 1928). In hexaploid oats, however, PHILP (1935, 1938) found two nullisomics which appeared in frequencies of up to 85 percent among the offspring of monosomics.

The relatively few data available on the separate male and female transmission of whole-chromosome deficiencies suggest that female transmission is about 75 percent, regardless of the chromosome concerned, and that male transmission varies from approximately 1 to 15 percent, depending on the chromosome. On the female side, 39 gametes analyzed from monosomic II showed 74 percent to be deficient, 32 from III showed 81 percent deficient, 20 from XV showed 70 percent deficient, and 23 from XVI showed 70 percent deficient. On the male side, 57 gametes from mono-I showed 5 percent deficient, 26 from III showed 19 percent deficient, and 23 from XVI showed 9 percent deficient. For the chromosomes concerned, these amounts of male transmission, coupled with about 75 percent female transmission, account reasonably well for the observed frequencies of nullisomics.

Data available on the proportion of monosomics among the non-nullisomic

plants in the progenies of monosomics tend to bear out the conclusions reached concerning transmission. The proportion of monosomics has varied approximately from 75 to 85 percent, with the higher values attributable to a selection of monosomics over normals for examination.

The transmission data agree reasonably well with those of NISHIYAMA (1928), who found 73 and 11 percent of functioning deficient female and male

TABLE 2

Chromosome constitution of the offspring of nulli-III plants pollinated by normals.

CONSTITUTION		NO. PLANTS OF SPECIFIED CONSTITUTION			
NO. MONOSOMES (IN ADDITION TO MONO-III)	NO. TRISOMES	IN POPULATION 1	IN POPULATION 2	IN POPULATION 3	IN POPULATION 4
0	0	54	44	45	74
1	0	24	17	25	18
2	0	4	1	6	2
3	0	0	1	2	0
0	1	9*	5	3	2
0	2	3	0	1	0
0	3	1	0	0	0
1	1	1	1*	1	1
1	2	0	0	2	0
2	1	0	1	3	0
2	3	0	0	1	0
3	2	0	0	1	0
Total		96	70	90	97
Inviable seeds		6	19	6	10

* Includes one secondary trisome.

gametes, respectively, from monosomic-"g" plants. From monosomic "f" he reported 71 and 37 percent of deficient female and male gametes, but the figure for male transmission is probably too high, for only 14 percent of nullisomics were obtained from selfing monosomic-"f" plants.

No evidence has been found of any selection against n−1 female gametes, such as was observed by GREENLEAF (1941) in *Nicotiana Tabacum*.

The preponderance of deficient female gametes is attributable to the frequent elimination of the univalent monosome through its failure to be included in a daughter nucleus at the reduction division. The low number of functioning deficient male gametes is presumably due to the considerable elimination of deficient pollen through competition with normal pollen.

The origin of monosomes from nulli-III plants

As mentioned previously, nullisomic-III plants are characterized by the presence in most MI microsporocytes of two or more univalent chromosomes. As shown in figure 1, there seems to be a general reduction in chiasma fre-

quency. Despite this abnormality, however, fertility is good, sometimes, reaching 100 percent in the more vigorous spikes. Functioning female gametes, all of which are of course deficient for chromosome III, may carry no additional abnormalities or may be deficient for as many as two or three additional chromosomes and duplicated for two or three others. Table 2 shows the constitution of the offspring of nulli-III plants pollinated by normals in four dif-

TABLE 3

Frequency of various monosomes in the progeny of nulli-III plants.

MONOSOME	FREQUENCY IN POPULATION 1	FREQUENCY IN POPULATION 2	TOTAL FREQUENCY
I	0	1	1
II	4	1	5
IV	12	2	14
V	0	1	1
VI	2	0	2
VII	2	1	3
IX	1	4	5
X	1	0	1
XI	0	1	1
XV	4	6	10
XVII	2	1	3
XVIII	3	0	3
XIX	0	2	2
XX	0	2	2
Lost	1	1	2
Unidentified	1	2	3
Totals	33	25	58

ferent seasons. Among 353 plants grown, 136 were aberrant. These 136 plants had 138 monosomes and 47 trisomes.

It appears from table 2 that the frequency of aberrant plants among the offspring of nullisomic III may vary from season to season. Population 4 had only 23.7 percent aberrant plants, while the average for the other three populations was 44.1 percent. This accords with the observation that the frequency of univalents in nulli-III plants is sometimes relatively low, particularly in exceptionally vigorous plants.

There also appears to be a tendency for certain chromosomes to occur unduly frequently as monosomes among the offspring of nulli-III. This is shown in table 3, which gives the frequency with which the various monosomes occurred in populations 1 and 2. Monosome IV occurred 14 times and mono-XV occurred ten times in the total of 58 monosomes in the two populations. Since there are 20 possible monosomes (excluding III), the mean frequency is 2.9 $\pm$ 1.66. Mono-XV therefore deviated from expectation by about four times the standard error, and mono-IV by more than six times the standard error—both highly significant values.

The high frequency of occurrence of monosomes IV and XV in offspring of nulli-III seems best attributed to a correspondingly frequent occurrence of these chromosomes as univalents in nulli-III. Presumably the tendency to pair may be affected by chromosome length, but other factors must operate as well. Chromosome XV is very short, but so is XIX, which occurred only twice. Whatever the factors determining which chromosomes shall pair when there is a tendency for a reduced frequency of chiasmata, such as in nullisomic III, the variation thereby effected would be expected to cause some monosomes to arise with unduly high frequency and others with unduly low frequency. Numbers are too small as yet to show whether or not the latter class exists.

TRISOMES AND TETRASOMES

Duplications as a rule affect viability less than do deficiencies, and it is therefore not surprising that $2n+1$ types have been found in various species much more frequently than have $2n-1$ types. Similarly, tetrasomic types ($2n+2$) are apparently more easily obtained than nullisomics. Six of the twelve possible tetrasomics in *Nicotiana sylvestris* have been isolated (GOODSPEED and AVERY 1939), while nullisomics apparently cannot be obtained either in this species or in the more highly polyploid species *N. Tabacum*, which is evidently an amphidiploid of *N. sylvestris*×*N. tomentosa* (CLAUSEN 1941b).

In wheat, tetrasomics have not been reported as often as nullisomics, presumably because they and the trisomic types from which they are derived occur less frequently than nullisomics and monosomics and have not been so easily distinguished phenotypically from normal. WINGE (1924) reported that homozygous speltoids were tetrasomic for one chromosome and nullisomic for another. HUSKINS (1928) described a similar situation for certain homozygous speltoids and reported others which were simply tetrasomic. BYNOV (1938b) found 44 chromosomes in homozygous speltoids, these presumably being tetrasomic. A short-compactum type was reported by UCHIKAWA (1937) to be tetrasomic. HUSKINS (1931) found that the unfixable dwarf of VILMORIN (1913) was trisomic and that the occasional extreme dwarf offspring were tetrasomic. Possibly the dwarfs reported by some of the other early investigators were also trisomic or tetrasomic types.

From the five trisomes present in the progeny of the monoploid, and from the numerous additional ones obtained from nulli-III (table 2), 11 tetrasomes have been obtained. The six of these which have been tested have proved to involve six different chromosomes, but it is probable that there are one or more duplicates of these among the remaining five. Only one has been identified with respect to the chromosome concerned, and that one involves chromosome II.

In general the tetrasomic plants are of more nearly normal phenotype than are nullisomics. Only one is of conspicuously reduced vigor and fertility, and that one is both male and female fertile. The fertility of tetra-II is somewhat reduced, but not nearly to the degree of nulli-II, which is female sterile. Tetra-II differs from normal in the opposite direction from nulli-II in most respects:

It tillers more than normal, has narrower culms and leaves, and has longer awns (fig. 2g).

Although tetrasomic plants frequently have quadrivalent configurations at meiosis, chromosome distribution is fairly regular. Among 44 offspring from six tetrasomics of different origin, all but eight were tetrasomic.

Most of the trisomics are little different from normal, except tri-II, which has the characteristics of tetra-II, but to a lesser degree. Tri-IX has tentatively been identified as a type with compact spikes.

The frequency of tetrasomic plants obtained among the offspring of trisomics has been similar to that of nullisomics in progenies of monosomics—that is, from about one to ten percent. It appears, however, that the extra chromosomes are transmitted through fewer female gametes and more male gametes than are the various deficiencies. Perfectly regular meiotic behavior of a trisome can result in only 50 percent of the gametes having an extra chromosome, and irregularities reduce this percentage by causing chromosome losses. The data thus far available indicate a frequency of 40 percent or less of disomic female gametes. With this comparatively low female transmission, male transmission must be somewhat higher than for the deficiencies.

COMPENSATING NULLISOMES AND TETRASOMES

Two instances have been found where a particular tetrasome cancels in part or almost entirely the effects of a certain nullisome, and one compensating trisome-nullisome combination has also been found. Similar compensating combinations have been reported by HUSKINS (1941) in both wheat and oats.

The combination tetra-II, nulli-XX is very nearly normal in all respects (fig. 2e), including fertility, although both tetra-II (fig. 2g) and nulli-XX (fig. 2a) are markedly abnormal. The compensation apparently extends to the male gametophyte, for preliminary data indicate no competitive advantage for normal pollen over pollen duplicated for chromosome II and deficient for chromosome XX. Of 39 offspring of a tri-II, mono-XX plant, none were of normal constitution, eight were tetra-II and nulli-XX, and the rest were of such constitution as not to permit identification of the gametes which produced them. Assuming that 75 percent of the germ cells were deficient for chromosome XX and that 36 percent were duplicated for II, 27 percent would be both deficient-XX and duplicated-II, and 16 percent would be normal. The other two types of gamete, which are not involved in the production of the critical plants, may be omitted from consideration on the male side, since presumably they are largely eliminated by pollen competition. In the absence of any competitive advantage for normal pollen over the duplicated-deficient type, six percent normal plants and 17 percent nulli-XX, tetra-II plants would then be expected—approximately a 1:3 ratio. An advantage for normal pollen would increase the proportion of normal plants. The fact that no normal plants and eight nulli-XX, tetra-II plants occurred out of 39 conforms reasonably well to expectation on the basis of no advantage to normal pollen. The deviation, in fact, is in the opposite direction, though not significantly so.

The ability of tetra-II to compensate for nulli-XX suggests that these two chromosomes have one or more segments in common. Further evidence of this is the fact that mono-II, mono-XX plants resemble the parental nullisomics in all respects, including infertility. The common segment is evidently not long, however, for no pairing at meiotic metaphase has been observed between monosomes II and XX or between trisome II and monosome XX.

The other compensating nullisome-tetrasome combination involves nulli-XVI and an unidentified tetrasome. The degree of compensation is not sufficient to result in normal plants, but it does decidedly improve the vigor and fertility of nullisomic-XVI. As with the II–XX combination, no tendency has been found for the two chromosomes to pair. Deficient-duplicated pollen apparently competes rather favorably with normal pollen. Of 15 male gametes from a monosomic-trisomic plant tested in a cross with a normal plant, six were deficient-duplicated and nine were normal. Comparison of this 6:9 distribution with the approximate 9.4:5.6 ratio expected in the absence of selection shows a deviation toward selection of normal pollen, but this deviation is not significant statistically.

The nullisome-trisome combination mentioned involves nulli-I and shows nearly perfect compensation. The combination originated when the trisome arose in a nullisomic-I line. No offspring have been grown.

Because of the favorable competitive position of $n-1+1$ pollen in which the duplicated chromosome tends to compensate for the missing one, there is selection in favor of compensating combinations. Hence, compensating nullisome-tetrasome combinations tend to appear with undue frequency. Several non-compensating combinations, however, have been obtained by crossing nullisomics with tetrasomics.

TELOCENTRICS AND ISOCHROMOSOMES

Recent work of UPCOTT (1937) and DARLINGTON (1939) has shown that univalent chromosomes, through misdivision of the centromere, may give rise to telocentric chromosomes, which are completely deficient for one arm, or to isochromosomes, which are deficient for one arm and duplicated for all of the other arm. Secondary aberrations of both these types occur in the offspring of trisomic and particularly of monosomic plants of *Triticum vulgare*. Either a telocentric or an isochromosome or both has been obtained for one or both arms of 15 of the 17 identified monosomes.

The frequency of telocentrics and isochromosomes among the offspring of certain monosomics appears to be rather high. For example, among 146 daughter plants of monosomic-VI grown in the 1940–41 season, two had a telomonosome and three had an isomonosome. In all five of these plants, which closely resembled nullisomics, the same arm of chromosome VI was involved. Since only 15 plants, mostly resembling nullisomics, were examined cytologically in this population, it is possible that several other individuals possessed telo- or isomonosomes for the other arm of VI, or telocentrics or isochromo-

somes for either arm in company with a normal chromosome. All of these types would presumably have been practically normal in appearance.

It is probable that the frequent occurrence of telocentrics and isochromosomes in the progeny of monosomics results primarily from pollen competition rather than from a high frequency of misdivision. Normal pollen has a strong competitive advantage over nullisomic pollen, and pollen deficient for only part of a chromosome should have a somewhat similar advantage, particularly if the majority of genes affecting gametophytic development happened to be located on the arm which was present. This selective advantage dependent on the proportion of important genes on the remaining arm would account for the differing frequencies with which telocentrics and isochromosomes involving different chromosomes and different arms of the same chromosome appear. It is not unlikely, however, that differences in the frequency of misdivision also occur.

The behavior of telocentrics and isochromosomes at meiosis and mitosis will be the subject of a subsequent paper. It may be stated here, however, that telocentrics in *T. vulgare* are sometimes lost in somatic tissue and that isochromosomes apparently may lose one arm or be entirely lost. RHOADES (1940) found that a telocentric chromosome in maize was occasionally lost or modified in somatic tissue, but that the corresponding isochromosome apparently behaved normally.

DISCUSSION

Although *Triticum vulgare* is an allo- rather than an autopolyploid, there is evidently considerable duplication in its chromatin. Numerous sets of duplicate factors are known; and STADLER (1929) found fewer mutations following X-ray treatment of the hexaploid *T. vulgare* than following treatment of diploid and tetraploid wheats, indicating that many gene loci are covered up by duplicate genes at other loci. Cytological evidence of duplication is also available, in that up to five pairs of chromosomes may occur at meiosis in monoploids.

There is as yet little indication as to how large a fraction of the total amount of chromatin is duplicated, nor how this duplicated chromatin is distributed among the chromosomes. The cytological data from the monoploids, where frequently no pairs and usually not more than one are found, show that no two chromosomes are homologous throughout their length and suggest that there is altogether little duplication. However, there is reason to believe that cytological data cannot be depended on to reveal inter-chromosomal homologies which involve short segments of chromatin. Several instances are known in which a tetrasome is able to compensate partially for a certain nullisome, but no tendency has been detected for the presumably homoeologous chromosomes to be associated at meiosis. Furthermore, the viability of the various nullisomic types suggests that the amount of duplication which exists may be much greater than indicated by the pairing in monoploids. Obviously

the nullisomics would not be viable unless every essential gene were duplicated in other chromosomes.

In *Nicotiana Tabacum*, an allotetraploid with 24 pairs of chromosomes, nearly all of the possible different monosomics have been obtained and several of these have been rather extensively tested, but none has given rise to a nullisomic (OLMO 1935; CLAUSEN 1941a, b). Similarly, LAMMERTS (1932) was unable to obtain the nullisomic types from seven different monosomics in *N. rustica* (n=24). The failure of nullisomics to appear in these two Nicotiana species is evidently due primarily to inviability of nullisomic zygotes, since several types of 23-chromosome pollen have proved functional to some extent. The contrast with *T. vulgare* is explainable by the fact that *vulgare* is a hexaploid rather than a tetraploid like tobacco, and therefore presumably has more duplication. In connection with this problem, certain of the deficiencies in *T. vulgare* are being transferred to a tetraploid wheat, where their effects both as monosomes and nullisomes (if obtainable) can be compared with their effects in the hexaploid.

For the genetic analysis of polyploid species, the nullisomic method is highly advantageous. Dominant or partially dominant factors, whether previously known or not, can be located on the chromosomes simply by observation of nullisomic plants. Recessive factors can be located by crossing each nullisomic to a strain carrying the dominant allele and observing the F_2's for departures from a 3:1 ratio. The critical F_2 will show the recessive condition only in the occasional nullisomic plants. Where ratios tend to be obscured by the segregation of duplicate factors or modifiers, or where classification is difficult, backcrosses of the F_1 can be made to the respective nullisomics until in each case all but the desired one of the chromosomes of the variety being tested are eliminated. The effects of the various chromosomes can then be compared against a uniform background. This method should prove particularly suitable for the location of factors for resistance to certain diseases, where reactions of individual plants may not be entirely trustworthy.

The chromosomal aberrations also yield information as to the action of certain genes. For example, the factor *B2* on chromosome X has been considered a dominant inhibitor of awns, because in most crosses *B2b2* resembled *B2B2* more closely than it did *b2b2*. This fact, however, fits equally well the theory that *B2* is without effect and *b2* promotes awn development, with a threshold effect such that two doses of *b2* result in awns more than twice as long as does one dose of *b2*. But the nullisomic decides in favor of the inhibitor hypothesis, since nulli-X is awned. The comparable gene *b1*, located on chromosome IX, even has a slight inhibitory action on awn development, as shown by the somewhat longer awns of nulli-IX than of *b1b1*.

Where both the nullisomic and the tetrasomic have been obtained for a particular chromosome, as for chromosome II, a series is made available in which the chromosome is present in doses of from 0 to 4, inclusive. Such a series may be used advantageously to measure the dosage effect of genes located on that chromosome.

SUMMARY

Seventeen of the 21 possible nullisomics in *Triticum vulgare* var. Chinese Spring have been obtained. Eleven of these (designated I to XI) involve chromosomes homologous to those of the emmer or tetraploid wheats, while the remaining six (XV to XX) involve chromosomes not represented in the emmers. All are reduced in vigor to various degrees, but all mature, and none is completely sterile. Nulli-III is partially asynaptic and has been used as a source of additional aberrations.

The location of several genetic factors is revealed by the nullisomics: A factor for red seeds on chromosome XVI, the hooded factor (*Hd*) for recurved awns on VIII, the awn suppressor *B2* on X, and factors for promotion of awn growth on II and XX. The speltoid chromosome, with factors for pubescent nodes, squareheadedness, and suppression of awns, is IX. The recessive *b1* on chromosome IX, whose dominant allele inhibits awns, also inhibits awns to a small extent.

Nullisomics have been recovered in frequencies of less than one to more than ten percent in the progenies of monosomics. The differences depend mainly on the elimination of different proportions of the deficient pollen. Apparently about 75 percent of functioning female gametes are deficient regardless of the chromosome concerned.

Tetrasomics have occurred among the offspring of trisomics in frequencies of about one to ten percent. One tetrasome involves chromosome II and compensates almost completely for nullisome XX. Another tetrasome compensates partially for nulli-XVI, and a certain trisome largely compensates for nulli-I.

Telocentrics and isochromosomes occur fairly frequently among the offspring of monosomic plants, following misdivision of the univalent chromosome.

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SEGMENTAL INTERCHANGE IN MICE

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INTRODUCTION

THREE lines, A, B and T, showing reduced fertility, were produced in mice by X-rays. Breeding experiments have shown that this X-ray-induced low fertility behaves as a dominant character which is most plausibly interpreted as due to structural rearrangement in the chromosomes. The suggestion that inherited reduced fertility was due to segmental interchange (reciprocal translocation) between two non-homologous chromosomes was first put forward by BELLING (1925) to explain some results obtained in species crosses of Stizolobium. The genetical implications of both spontaneous and induced segmental interchange have been analysed cytologically and genetically in various plants—namely, Datura (BLAKESLEE 1929), Zea (BURNHAM 1930, 1932, 1934), Pisum (SANSOME 1931, 1933; SUTTON 1935), and in Drosophila (DOBZHANSKY and STURTEVANT 1931). In Zea and Pisum the interchange heterozygote has been referred to as "semi-sterile" due to the fact that in these plants nearly all the interchanges are reported to be associated with 50 percent sterility.

SNELL (1933) was the first to report X-ray-induced "semi-sterility" in mice. This was followed by the discovery that reduced litter size of the interchange heterozygote is due to zygotic lethality (SNELL, BODEMANN, and HOLLENDER 1934; SNELL and PICKEN 1935; BRENNEKE 1937). The manifestation and behaviour of X-ray-induced semi-sterility in mice has been most extensively studied by SNELL (1935) and HERTWIG (1935, 1938, 1940) in breeding experiments. The presence of a characteristic chromosome configuration during the meiotic division in the descendants of X-rayed mice showing low fertility was detected by KOLLER and AUERBACH (1941) and was assumed to be brought about by the association of two pairs of non-homologous chromosomes due to segmental interchange. The present paper contains a more detailed description of the cytological analysis of interchange heterozygotes in mice and discusses the causal relationship between low fertility and chromosome behaviour.

MATERIAL AND METHOD

The method of inducing and detecting reduced fertility or semi-sterility was the same as that used previously by SNELL (1935), A number of males from stocks kept in this Institute had their testes exposed to X-ray doses varying between approximately 750 and 900 r. From three of these males semi-sterile lines were obtained, indicating the production of segmental interchange by the radiation. Average litter size in the stocks from which the males were taken, and from which later on normal animals were chosen for fertility tests, was 7.7. The three interchange lines A, B, and T were kept going by selecting in every generation a certain number of semi-sterile animals (male or

female), either by subjecting them to breeding tests or, in the case of males, by cytological examination of one testis, leaving the other in position and functioning. Once it had been shown that the results of the two methods were in good agreement, the cytological test was preferred because it saved labour and time.

Small pieces of testis were fixed in modified Minouchi's solution (2 percent osmic acid, 1 percent chromic acid, 3 percent potassium bichromate, and distilled water, in the ratio 2:4:4:10). Sections were cut at 16–18μ thickness and stained with gentian violet. Squash and smear preparations were also made; they were fixed in acetic alcohol (3:1) stained with lacmoid, orcein, or Feulgen's basic fuchsin (DARLINGTON and LA COUR 1942).

Living spermatozoa of normal and interchange heterozygotes were taken from the epididymis and studied by dark field illumination.

CHROMOSOME MORPHOLOGY IN THE MOUSE

The accounts given by various authors concerning the mitotic chromosomes in the mouse are in agreement that the diploid number is 40 and that the chromosomes are "rod-shaped" (cf. CREW and KOLLER 1932; MAKINO 1941). Improved methods of fixation and staining, however, showed that the apparently "rod-shaped" chromosomes are composed of one long and one very short arm, which indicates that the centromere is not terminal. In some chromosomes its subterminal position was clearly seen (fig. 1). The structure and shape of

FIG. 1. The diploid chromosome complement in the spermatogonium of the mouse. Squash preparation, stained with orcein. ×1,200.

bivalents during the first meiotic division also show that the chromosomes of the mouse are not "telomitic" or telocentric. They form a graded series in regard to length but may be arbitrarily classified into three groups consisting of large, medium, and small chromosomes. The shortest member of the diploid complement is about one-third the length of the longest chromosome. The two sex chromosomes in the male are of unequal length; the X is apparently one of the largest chromosomes, while the Y belongs to the smallest chromosome group. The unequal or "heteromorphic" sex bivalent can easily be identified during the meiotic division (plate 1, I).

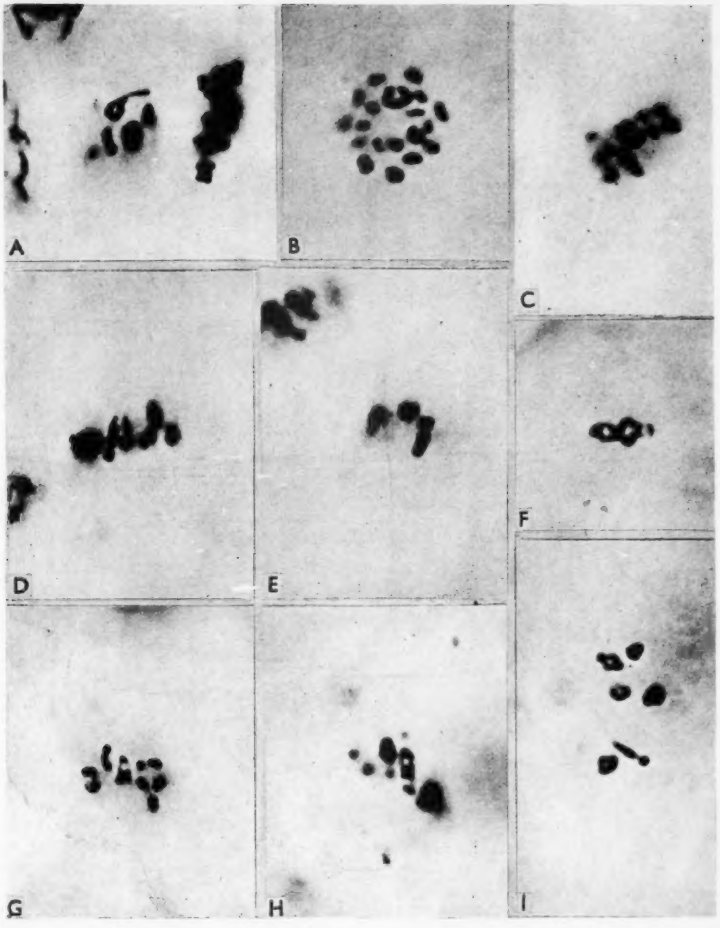

PLATE 1.—A. Trivalent, simulating a "triple chiasma" in a primary spermatocyte of line B. ×1,100.—B. Ring-of-four association in line T, showing non-disjunctional co-orientation of chromosomes. ×1,100.—C. Unequal bivalent in line B, (early anaphase). ×1,100.—D. Unequal bivalent in line A. ×1,100.—E. Unequal bivalent in line B. ×1,100.—F. Figure-of-eight association in line B. ×1,430.—G. Trivalent in line A. ×955.—H. Trivalent in line B. ×955.—I. The unequal sex chromosomes forming a bivalent. ×1,100.

CHROMOSOME BEHAVIOUR AND SEGMENTAL INTERCHANGE

Line T

The chromosomal basis of reduced fertility was most extensively studied in line T both cytologically and by breeding experiments. The number of first

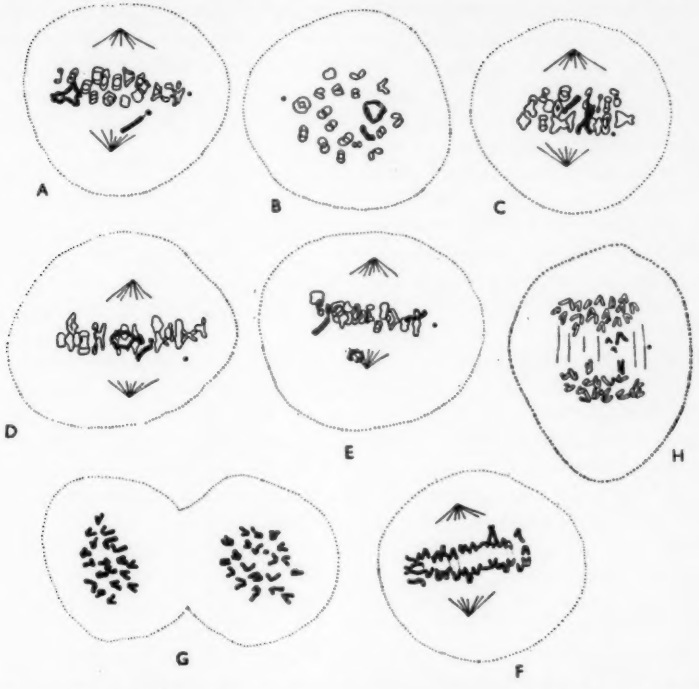

FIG. 2. Chromosome behaviour in the primary spermatocytes of interchange line T. The unequal sex bivalent and the chromosomes involved in the segmental interchange are drawn solid black. Description of figures in text. ×1,000.

generation offspring of semi-sterile T's tested was 26; 11 were found to be fertile and 15 semi-sterile—that is, about half of the viable offspring of semi-sterile parents carry the interchange. Similar results were reported on a much larger scale by SNELL (1935) and Hertwig (1940). The chromosome behaviour was studied in the primary spermatocytes of eight semi-sterile males. In many cells besides the 18 bivalents, including the unequal XY chromosome pair, a large ring representing an association-of-four was clearly seen (Plate 1B, text fig. 2B). As a result of reciprocal interchange of non-homologous chromosome segments, a ring-of-four is brought about by pairing and crossing over of one chromosome with a second chromosome at one end, and with a third at the other (*cf.* DARLINGTON 1937). Associations-of-four resembling a "figure-of-eight" configuration were also observed (fig. 2A). This configuration is the

result of five crossovers, one of which has taken place in an interstitial segment —that is, in the chromosome region between a centromere and the point of exchange,—while the four others are formed in the interchange segments and in the short arms. This configuration should be distinguished from the true figure-of-eight in which six chromosomes are associated (SANSOME 1933).

A cross-shaped configuration was also encountered (fig. 2C), and it was assumed that the association of the four chromosomes is the result of three chiasmata, one in an interstitial segment and one in each of the two interchanged chromosome arms. In a few primary spermatocytes the four chromosomes formed a chain; this configuration is also due to three chiasmata, formed in three chromosome arms.

Primary spermatocytes were also found with 20 bivalents. While the unequal XY bivalent was easily recognized, no unequal bivalents were identified (fig. 2F). In a few cells a chromosome configuration of an unusual shape was observed; it may represent the interchanged chromosomes forming a "bivalent" (fig. 2E). The new chromosome types could not be identified in the diploid complement, which suggests that the interchanged segments themselves are of similar size.

The anaphase segregation of chromosomes from an association-of-four is determined by their co-orientation during metaphase. This may be either (1) disjunctional—that is, adjacent members move to the opposite poles (convergent or zigzag arrangement), (2) non-disjunctional (fig. 2D), or (3) chromatid non-disjunctional (which results from the figure -of-eight). The non-disjunctional (linear, parallel, or discordant) co-orientation necessarily leads to the formation of genetically unbalanced gametes (DARLINGTON 1937). Chromatid non-disjunction or half disjunction leads to the production of half balanced, half unbalanced gametes. The frequencies of the first two kinds of chromosome co-orientation in the ring-of-four associations of line T were found to be very nearly the same.

Besides the two-by-two non-disjunctional arrangements, numerical non-disjunction, by which three chromosomes went to one nucleus and one to the other, was observed (fig. 2H, G). The relative frequencies of these two types could not be determined.

The analysis of the type and the frequency of the various multi-chromosomal associations strongly suggest that, in semi-sterile line T, two non-homologous chromosomes of medium size interchanged relatively short segments of similar lengths.

The total number of primary spermatocytes analysed in the eight males of line T was 229 (86, 31, 25, 23, 20, 17, 15, and 12 in the different animals). The data obtained show that in the different males the frequency of the same chromosome associations and consequently the frequency of the disjunctional and non-disjunctional gametes is very similar.

Line B

Chromosome behaviour during the first meiotic division was characterized by a frequent failure of chromosome association. Univalents were often en-

countered in primary spermatocytes. The unpaired chromosomes could easily be identified by their size and shape and by the number of bodies present in the primary spermatocytes (fig. 3F, H). It was found to be 22 ($18^{II}+4^{I}$) or 21 ($19^{II}+2^{I}$). In some cells only two univalents were seen; the other two chromosomes forming an unequal pair could not be identified (fig. 3G). The failure of pairing frequently observed in the primary spermatocytes is attributed to the interchanged chromosomes.

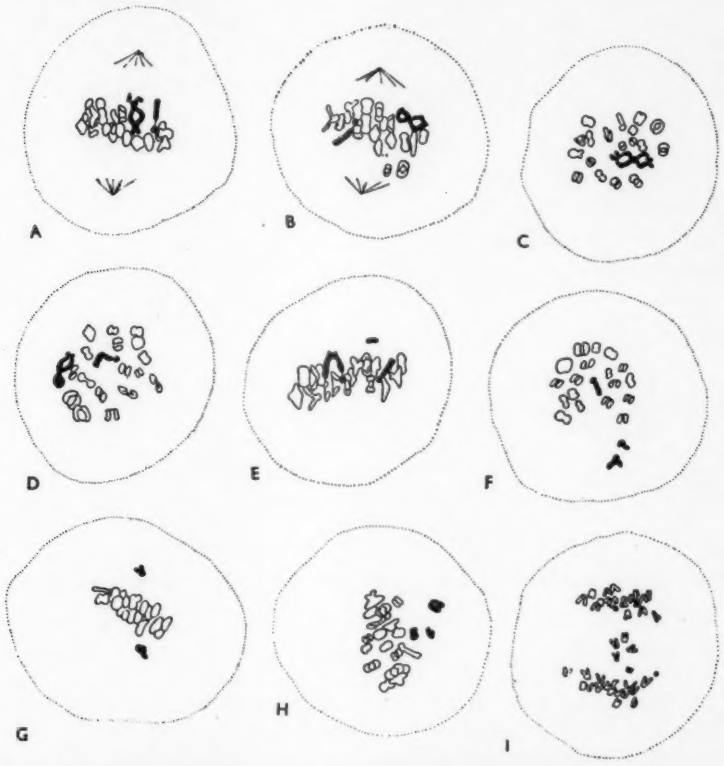

FIG. 3. Chromosome behaviour in the primary spermatocytes of interchange line B. ×1,000.

Another feature of chromosome behaviour in line B is the high frequency of the zigzag configuration (fig. 3A–D) as compared with that found in lines A and T. Many of the zigzag configurations are very similar to the figure-of-eight (Plate 1F), because the overlapping of chromosomes may easily be mistaken for a chiasma in an interstitial segment. Owing to the difficulty in distinguishing chromosome overlapping from a true chiasma, it is more than probable that the frequency of disjunctional co-orientation in rings-of-four of line B as it is given in table 3 is an underestimate.

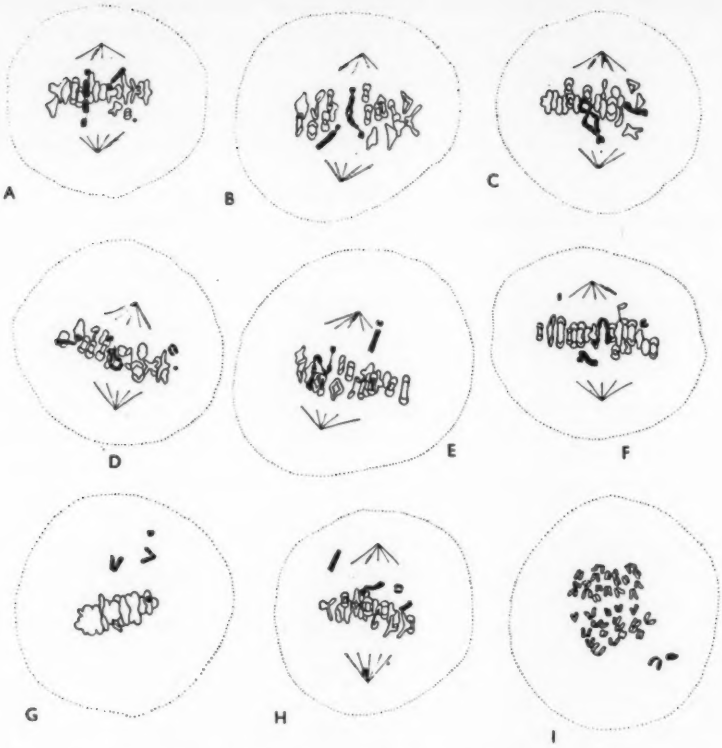

FIG. 4. Chromosome behaviour in the primary spermatocytes of interchange line A. ×1,000.

In line B, chains-of-four chromosomes were not seen; an association-of-three chromosomes was observed in about 7 percent of the primary spermatocytes analysed (fig. 3E; Plate 1A, H). Unequal bivalents were also identified (Plate 1C, E); they differ from the XY (Plate 1I) and may be easily distinguished. Evidence of numerical non-disjunction was also obtained (fig. 3I).

The types and frequencies of various chromosome associations indicate that the chromosome pairs involved in the interchange of line B are of medium size and that the interchanged segments differ greatly in length. Due to this difference, the unequal bivalents could easily be distinguished.

Chromosome behaviour was analysed and found to be similar in five semi-sterile males. The total number of primary spermatocytes studied was 156 (68, 25, 23, 20, 20).

Line A

It was found that in the primary spermatocytes of line A the chain-of-four chromosome association showing linear or non-disjunctional co-orientation was

TABLE 1

Litter size of interchange heterozygotes in lines A, B, and T.

LINE	SEX	GENERATION	NO. OF YOUNG IN SUCCESSIVE LITTERS	MEAN LITTER SIZE	NOTES
		F_2	2, o*, o*, o*	—	C.A.** 2 V.P.†
		F_2	o*, o*, o*, o*	—	C.A. 2 V.P.
	♂♂	F_2	o*, o*	—	2 V.P.
		F_2 (TA8:2)	3, 4, 1, 3, 5, 6	3·2	—
A					
		F_1	1, 2, 4, 2, 4	2.6	—
	♀♀	F_2	2, 2, 2, 2, 6	2.8	—
		F_1	6, 4, 3, 6, 6, 2	4·5	C.A.
		F_2	4, 4	4.0	C.A.
	♂♂	F_2	4, 3, 6	4·3	C.A.
		F_3	5, 3, 3	3·7	C.A.
		F_4	5, 1, 4	3·3	C.A.
B					
		F_2	1, 3	2.0	
		F_2	2, 3, 2	2.3	
	♀♀	F_2	3, 4	3·5	
		F_3	3, 3	3.0	
		F_4	3, 4, 2	3.0	
		F_1	2, 3, 2, 2, 1, 2	2.0	C.A.
		F_2	2, 3, o*, o*, o*, o*		C.A. 1 V.P.
		F_2	3, 2, 4, 3, 5, 4	3·5	C.A.
		F_2	2, o*, o*, o*, o*		C.A. 2 V.P.
	♂♂	F_2	3, 5, 6, 3	4·3	
		F_2	5, 6, 3, 4	4·5	
		F_2	5, 3	4.0	
		F_3	4, 3, 5	4.0	
T					
		F_2	2, 4, 6	4.0	
	♀♀	F_2	3, 2, 4, 5, 6, 5	4.1	
		F_2	5, 5, 7, 2, 2	4.2	
		F_2	4, 1, 3	2.7	

* o = No litter from mating with separate females.
** C.A. = Cytologically analysed.
† V.P. = Vaginal plugs observed.

very frequent (fig. 4A, B). Configurations, very similar to a figure-of-eight with non-disjunctionally arranged chromosomes, were often encountered (fig. 4C). Chains with disjunctional co-orientation are relatively rare (fig. 4E). Associations-of-three (fig. 4F; Plate 1G) and unequal bivalents (Plate 1D) were also observed. Spermatocytes with univalents are frequent (fig. 4G, H). It is possible that failure of pairing leads to numerical non-disjunction (fig. 4I). A chromosome configuration resembling a trivalent of a secondary trisomic was seen (fig. 4D). Its true structure and origin could not be determined.

The analysis of chromosome association suggests that one medium and one short chromosome pair are probably involved in the interchange of line A. The interchanged segments are unequal. It is believed that one of the breaks occurred in the long, the other in the short arm of two non-homologous chromosomes. The frequent failure of pairing indicates that the unequal interchanged segments are relatively small, their length is below or near the minimum length which conditions chiasma formation.

Four males of line A with low fertility were investigated. The number of primary spermatocytes analysed was 172 (73, 52, 30, and 17). The frequencies

TABLE 2

Mean litter size and fertility in the interchange lines A, B, and T.

LINE	NO. OF LITTERS	MEAN NO. OF OFFSPRING IN LITTER	FERTILITY (PERCENTAGE)
Control		7.7	100
A	23*–17	2.3–3.0	29.9–39.0
B	29	3.5	45.7
T (F_1, F_2, F_3)	48*–45	3.4–3.6	43.6–46.5
T (F_3)	16	3.9	51.2

* Figure based on the inclusion of sterile matings as determined by the observation of a vaginal plug.

of the various chromosome configurations were similar in all the males except one.

Line A was found to be the most sterile. On account of the sterility of several males it was at first thought that the sex chromosomes might have been involved in the interchange. However, breeding tests and cytological analysis have shown that this is not the case. The sex bivalent (Plate 1I) differs from the unequal bivalent (Plate 1D) formed by the interchanged chromosomes.

DIFFERENTIAL FERTILITY BETWEEN INTERCHANGE LINES

Breeding experiments have shown that the three interchange lines differ significantly in fertility, measured by the litter size. The results are summarized in tables 1 and 2. Animals producing only one litter are not included.

At the onset of the investigations the breeding tests for fertility were carried out with the sole object of identifying semi-sterile individuals. When the cytological analysis was near its completion, a possible connection between the various degrees of infertility and types of chromosome configuration was suspected. At this time the bulk of the breeding had already been done, so that most of the given data on which estimates of litter size are based were collected at a time when there seemed to be no reason for particular caution. In order to assist future investigations of similar problems, it does not seem superfluous to point out some of the factors which may have been a source of error in estimat-

ing the degree of fertility in the different lines. (a) The size of the litters was not always recorded on the day of birth. On account of early depletion in certain litters, this may have reduced litter size slightly in both control and interchange lines. (b) Animals of the interchange lines which had in their first litter six or more young were not further tested; they were considered to be normal. When a surplus of animals was available, even first litters of five were taken as a basis for discarding an animal. This procedure will tend to reduce the figure for litter size in lines B and T, in which litters with five or six young were not infrequent. The breeding data from line A has presumably not been influenced by this source of error. In this line animals with a first litter of five were not discarded without further test on account of the small number of animals available for carrying on the interchange. Furthermore, litters in line A with more than four young were very rare and occurred only from the third generation onward. (c) Some males in line A and in the F_2 of line T produced either no litter at all, or one or two litters with one or two young, although they were kept with different females (renewed from time to time) for several weeks. In some instances, the presence of vaginal plugs was observed, indicating that mating had taken place but failed to produce a litter. These matings can probably be regarded as litters of size 0. Separate totals are calculated with these matings included. (d) The mean size of litters (7.7) in controls was computed from a random sample of litters produced during the first months of the experiment by animals from stock; the same stocks were used for outcrosses. The fertile sibs of semi-sterile animals were not usually kept for breeding; data based on their performance comprise mainly first and second litters and are therefore less reliable controls than those gained from the stocks.

As will be seen from the data summarized in table 2, line A showed about 30–39 percent fertility, line B about 46 percent fertility, line T about 44–46 percent fertility. Line A is probably significantly less than 50 percent fertile. In the case of lines B and T, the existence of a significant departure from 50 per cent fertility is uncertain. It is of interest to consider the cause of the different fertility of the three lines.

Random two-by-two separation of the four members of the translocation ring gives six types of gametes in equal numbers. The proportion of the genetically balanced and unbalanced gametes (2:4) in interchange heterozygotes is determined by the metaphase arrangements of translocation rings and may also be influenced by the formation of associations-of-three and univalents. Thus BRINK and COOPER (1933) found that 50 percent fertility in an interchange line (semi-sterile-1) of maize is associated with an equal frequency of non-disjunctional and disjunctional co-orientation of chromosomes in the ring-of-four. The two largest chromosome pairs are involved in this interchange.

Significant departures from 50 percent fertility occur. Thus BURNHAM (1930, 1934) reported an interchange in maize (semi-sterile-2) showing 41 percent fertility. In semi-sterile-2 rings are formed frequently, but chain configurations are more frequent than in semi-sterile-1. Numerical non-disjunction occurs. Anaphases have been counted in which there were eleven chromosomes at one

pole and only nine at the other. The resulting n+1 pollen is capable of functioning, as indicated by the fact that 21 chromosome plants are occasionally produced. The n−1 pollen presumably aborts and may account entirely or in part for the sterility in excess of 50 percent. Two of the shorter chromosomes are involved.

The importance of the length of the interchange segments as well as that of the chromosomes in determining co-orientation was proved by BROWN (1940) and PIPKIN (1940) in Drosophila; they corroborated the findings of DOBZHANSKY (1933) that the frequency of non-disjunction is negatively correlated with the length of the interchange segment. The number of chiasmata in an association-of-four is another factor which influences co-orientation. BEADLE (1932) reported in maize that fertility of an interchange heterozygote is increased from 43 percent to 48 percent when the ring-of-four association is replaced by the chain-of-four. A similar observation was made by SAX and ANDERSON (1933) in Tradescantia. Furthermore, the different fertility of interchange heterozygotes in Datura, Zea, Pisum, and Campanula indicates that the position and terminalisation of chiasmata also play a part in determining chromosome co-orientation in the multivalent (GAIRDNER and DARLINGTON 1930). In Datura and Campanula, also Oenothera, terminalisation is complete and the percentage of disjunction high, whereas in Zea and Pisum with 50 percent of non-disjunction, interstitial chiasmata are present at metaphase. Chromosomes with a median centromere form a disjunctional ring-of-four of the zigzag type more often than do chromosomes with a subterminal centromere. Thus the fertility of interchange heterozygotes is high in Datura, Oenothera, and Campanula, and much lower in Tradescantia and Rhoeo. According to BERGNER, SATINA, and BLAKESLEE (1933), most of the translocation heterozygotes are fully fertile in Datura. THOMPSON and HUTCHESON (1942) reported 95–90 percent fertility in diploid wheat. SAX and ANDERSON (1933) observed a very high frequency of non-disjunctional co-orientation (80 percent) in the ring-of-twelve of Rhoeo. In mice the fertility of the interchange line A is less than that observed in B and T, which indicates clearly that the structure and behaviour of the chromosomes involved in the interchanges differ in these lines and determine the different frequencies of balanced gametes.

Since anaphase segregation is determined by the metaphase association and co-orientation of the interchange chromosomes, if the frequencies of the various types of association and co-orientation are known, then the frequency of unbalanced gametes, and consequently the degree of fertility itself, can be calculated and predicted. The frequencies of disjunctional and non-disjunctional co-orientations, of figures-of-eight, of associations-of-three, of unequal bivalents, and of univalents in the three interchange lines are given in table 3. The litter size of those males whose chromosome behaviour was analysed was nearest to the mean litter size characteristic of the line; thus their chromosome behaviour may be considered to be representative for the particular interchange. The number of those spermatocytes in which the method of association of the interchanged chromosomes could not be determined is also given. The per-

TABLE 3

Fertility of the interchange lines estimated by the chromosome behaviour.

CHROMOSOME CONFIG.		PERCENTAGE OF BALANCED GAMETES	INTERCHANGE LINES		
			A	B	T
Ring-of-four	Disjunct.	100	2	8	8
	Non-disj.	0	5	4	16
Chain-of-four	Disjunct.	100	2	0	10
	Non-disj.	0	13	0	1
Figure-of-Eight	Normal	50	0	7*	12
	Abnormal	0	8**	4†	3
Association-of-three		33·3	2	3	0
Unequal Bivalent		50	10	12	9
Univalent		25	14	9	0
Non-analysed		—	17	21	27
Total no. primary spermatocytes		—	73	68	86
Fertility (percentage)	Expected (cytological)		23·5	44·2 or more	48·3
	Observed (breeding)		29·9–39	45·7	43·6–46·5

* Some are probably disjunction ring-of-four.

** See fig. 4C.

† See fig. 3C.

centage of these cells is 23.2 percent, 41.1 percent and 31.3 percent in line A, B, and T, respectively, which is very high and shows the great difficulty involved in an accurate cytological analysis.

A fairly satisfactory agreement between expected (cytological) and observed (breeding) fertility was found in semi-sterile line T; the fertilities are 48.3 and 43.6–46.5 percent, respectively. The four F_3 interchange heterozygote males analysed had a similar fertility calculated by chromosome behaviour.

In the case of line B the figures are 44.2 percent fertility (expected cytological) and 45.7 percent fertility (observed breeding). If allowance is made for chromosome configurations classed as figures-of-eight but perhaps actually disjunctional rings-of-four, the cytological fertility would be higher.

Line A has the most sterile interchange; fertility calculated from observed litter size is about 39 percent of that shown by the controls, or if sterile matings are included in the calculation, about 29.9 percent. This agrees well with the expected fertility of 23.5 percent calculated from cytological observations.

It should be pointed out that the data given above are only a very approximate estimate owing to the difficulties of cytological analysis. Chromosomes of the mouse are too small and numerous to enable very detailed and accurate observations to be made. The data which have been obtained are sufficient, however, to show that the differences in fertility between the lines are corre-

lated with the frequency of disjunctional co-orientation of chromosomes within the translocation ring and may also be influenced by differences in the frequency of associations-of-three and of univalents.

The three interchange lines show that radiation induces segmental interchanges which differ and that the differences may be detected not only by breeding but also by cytological analysis. There is evidence that in the similar experiments of HERTWIG (1940) different interchanges were produced by radiation. Thus she found that litter size is 4.05 in line 746 and 2.74 in line 621; in line 623 the embryos die late, about ten days after fertilization. HERTWIG suggests that

TABLE 4

Fertility of interchange heterozygotes in successive generations of line T.

GENERATION	NO. OF INTERCHANGE HETEROZYGOTES		NO. OF LITTERS	MEAN LITTER SIZE	FERTILITY (PERCENTAGE)
	MALE	FEMALE			
F_1	1		6	2.0	25.9
F_2	3	4	23	3.7	48.6
F_3	4	2	16	3.9	51.2

this line carries a "relatively small translocation" producing little unbalance in the gametes and allowing the embryos to develop to an advanced stage. It is probable that differences observed by her between the semi-sterile lines, involving litter size and time of zygotic lethality, are expressions of differences involving the interchanged chromosomes.

DIFFERENTIAL FERTILITY WITHIN INTERCHANGE LINES

In the third generation of the interchange heterozygotes of line T, a sudden increase was observed in fertility (table 4). It may be seen that while the F_1 male (progenitor of line T) exhibits a very low fertility (25.9 percent), in his offspring and descendants it has risen to 48.6–51.2 percent. Owing to the fact that, of the three F_2 males, two not only had a very small litter size, but also failed to produce litters with several females known to be fertile, the fertility of F_2 interchange heterozygotes is probably much less than 48.6 per cent. The litter size in F_3 may perhaps be considered to be most truly characteristic of interchange T, since other induced aberrations, if present, probably would have segregated out. Cytological analysis of the F_1 and F_2 males did not show any additional irregularities, except perhaps in one F_2 male in which a few spermatocytes were seen with univalents.

The data indicate that besides the segmental interchange, either minor, not detectable structural changes or gene mutations with deleterious effects on fertility were also brought about in the chromosomes by radiation and transmitted to the later generations by chance segregation. These additional changes, on account of the higher degree of sterility they cause when associated with the major chromosomal rearrangement, are rapidly eliminated.

Differential fertility was also encountered in line A. Three F_2 males when mated to fertile females failed to produce offspring. Vaginal plugs indicated that matings had taken place. An F_2 male was apparently more fertile. The loss of additional induced irregularities is perhaps responsible.

Similar cases of differential fertility within an interchange line have been described by other authors. CLARKE and ANDERSON (1935) found greater sterility in the earlier generations of an interchange in maize than in later generations. Completely sterile individuals were reported by HERTWIG (1940) amongst descendants of the semi-sterile male No. 733. They disappeared in the third generation. It is very probable that these mice were carrying additional chromosome changes or gene mutations induced by the radiation.

INTERCHANGE HOMOZYGOTES AND MULTIPLE INTERCHANGE HETEROZYGOTES

Several sister×brother matings of semi-steriles were attempted in order to produce interchange homozygotes in lines B and T. It was found that the number of offspring in this cross, as expected, fell below the mean number characteristic of lines T and B when outcrossed. Cytological analysis of 26 males in line T and 22 in line B has shown either normal chromosome behaviour or, besides the 18 bivalents, an association-of-four indicating the presence of an interchange in heterozygous condition. Some of these males which show 20 bivalents in every primary spermatocyte may have been homozygous for the interchange. Several such males were outcrossed, and the absence of quadrivalents suggests that they were normal. It is not improbable that the failure to detect interchange homozygotes in lines T and B may be due to the fact that the interchanges are lethal when homozygous. Owing to various difficulties arising from the war, the breeding experiments as originally planned could not be carried to completion, and the data obtained are not sufficient to draw a definite conclusion. In similar experiments HERTWIG (1940) reported two certain and one uncertain interchange homozygotes, which indicates that interchanges in mice as in Drosophila are not necessarily lethal when homozygous.

Another experiment was planned to produce multiple interchange heterozygotes by combining interchanges A and T in one individual. This could have been detected cytologically by the presence of either two rings-of-four or a ring-of-six if one of the chromosomes involved in the interchanges was common. The cytological analysis of offspring of 12 matings did not indicate the presence of these interchanges in combination. It was noticed by breeding tests that a great reduction was induced in fertility by crossing the two interchange heterozygotes together, the litter size falling below two. When two different interchanges are crossed, the expected ratio of viable to inviable zygotes is approximately 1:3.

FACTORS DETERMINING THE FREQUENCY OF UNBALANCED GAMETES

Breeding tests have shown that there is a difference in the degree of fertility (a) between the three interchange lines and (b) probably between some individuals within the same line. The factors which determine the frequency of the

non-disjunctional or genetically unbalanced gametes, and consequently the degree of sterility of an interchange heterozygote, may be classified as follows: (1) length (a) of the chromosome, (b) of the interchanged segments, (c) of the interstitial segment; (2) position (a) of the centromere, (b) of the chiasma; (3) number of chiasmata; and (4) degree of terminalisation.

The expression of sterility is the result of an interaction of these different factors. The variations in chromosome structure and behaviour underlie the difference in sterility found not only between the interchange heterozygotes of the different species, but between individuals carrying the same interchange, examples of which have been found in mice.

ZYGOTIC AND GAMETIC LETHALITY IN INTERCHANGE HETEROZYGOTES

In mice reduced fertility of the interchange lines is due to zygotic lethality. The motility and morphology of living spermatozoa in normals and interchange heterozygotes were studied by means of dark field illumination. A small portion (about 5–7 percent) of immobile spermatozoa was seen in both normals and interchange heterozygotes, due apparently to environmental factors. The genetically unbalanced spermatozoa of the latter could not be detected by this means. A more extensive comparative study of living spermatozoa of different interchanges would be very desirable in view of the fact that chromosome or genic unbalance may lead to the immobility of the sperm. That either genic or numerical unbalance may lead to gametic lethality in animals was shown by DETLEFSEN (1912). He reported the presence of immobile spermatozoa in the interspecific hybrids of *Cavia rufescens*×*C. porcellus*. The proportion of immobile sperm decreases in the successive backcross generations, due apparently to a reduction in the chromosome unbalance. There is evidence that in the interchange line A the differentiation of some spermatids into spermatozoa is arrested. These may represent the numerically unbalanced gametes. The elimination of unbalanced gametes at this early stage would, of course, raise the fertility as estimated by litter size.

The investigations of SNELL, BODEMANN, and HOLLENDER (1934), SNELL and PICKEN (1935), BRENNEKE (1937), and HERTWIG (1940) have shown that reduced litter size is brought about either (1) by the elimination of fertilised eggs before or after implantation or (2) by the death of the embryo during early or late development. When estimating sterility of interchanges in animals, it should be realized that eggs fertilised with unbalanced sperm may be eliminated before implantation, hence the number of "uterine moles" or dead embryos cannot be relied upon as the measure of reduction in fertility, for which reason in the present study this method was deliberately avoided. The stage in which lethality comes into operation is determined primarily by the degree of unbalance. It is not impossible that it may lead to the inactivity of spermatozoa or even arrest the differentiation of spermatids into spermatozoa.

On the one hand the possibility that the genetically unbalanced gametes may be viable in plants as in animals, and on the other, the fact that the egg in animals when fertilised by a greatly unbalanced gamete may be eliminated

in the first or second cleavage division suggest that the difference in the behaviour of non-disjunctional gametes in animals and plants is not so discontinuous as it appears to be. The occurrence of greater gametic lethality in plants, nevertheless, is to be expected because of the fact that they have a gametophyte stage, between meiosis and gamete production, during which two or three mitotic divisions occur. It is here, in plants, that unbalance usually leads to breakdown.

HERITABLE LOW FERTILITY IN DOMESTIC ANIMALS

The most significant genetical implication of segmental interchange is reduced fertility; because of this it is usually selected against in nature. However, instances are known, mostly in plants—namely, Oenothera—where heterozygotes are the rule. Amongst animals the best known interchange found in a free living population was reported by CAROTHERS (1931). Interchange, because it facilitates the establishment of a balanced lethal mechanism, can be maintained in nature and may lead to isolation and formation of incipient species. Comparative cytological analysis in closely related species (Drosophila, Datura, Crepis, etc.) has shown that interchange actually must have played a part in species differentiation. It may be fixed somewhat more easily in animal than in plant populations according to the mathematical calculation of WRIGHT (1941).

While an interchange, unless it is associated with a balanced lethal mechanism, is usually eliminated by natural selection, artificial selection in animals under domestication may favour its maintenance, especially if the chromosomes involved carry genes favoured in breeding. It is interesting to note that in domestic animals (cattle, horses) reduced fertility, often approaching complete sterility, is very common and is attributed to various causes—genic, physiological, nutritional, etc. In view of the fact that structural changes occur in the chromosomes of higher organisms, e.g., inversion in man (KOLLER 1937) and in the pig (CREW and KOLLER 1939)—it is not improbable that inherited reduced fertility in some of the highly infertile domestic animals may also be due to structural changes. It would be of great interest to analyse cytologically domestic animals known to be transmitting reduced fertility in order to determine whether or not this is due to segmental interchange.

The cytological analysis given in the present paper shows that radiation can induce heritable semi-sterility or reduced fertility in organisms higher than plants or insects. Nowadays, when radiation is extensively employed in radiotherapy, engineering, physics and chemistry, man is exposed to radiation in various ways. There can no longer be any doubt that the chromosomes of the germinal cells in man if not protected from X-rays may also undergo the same structural changes as in mice. The potential consequence is semi-sterility or reduced fertility in the descendants of the individual irradiated.

SUMMARY

Three interchange lines, A, B, and T, each from a different male, were produced by radiation (600–900 r). An association of four chromosomes, due to

segmental interchange between two pairs of non-homologous chromosomes, was identified during the first meiotic division in each of the lines.

The approximate fertility of the three lines estimated by breeding tests was found to be 30–39 percent in A, 46 percent in B, 44–46 percent in T (51 percent in the F_2 generation of T).

Cytological analysis has shown that differences in fertility are correlated with corresponding differences in the frequencies of non-disjunctional co-orientation of chromosomes in the ring-of-four. Fertility estimated on cytological analysis is expected to be about: 24 percent in A, 44+ percent in B, and 48 percent in T.

Differential fertility observed in successive generations within lines A and T is attributed to either minor structural changes or gene mutations brought about by the radiation responsible for the segmental interchanges.

The various factors on which depends the frequency of genetically balanced gametes—that is, the fertility of an interchange heterozygote—are analysed.

It is suggested that in some instances, inherited low fertility, common in domestic animals, may be due to segmental interchange, the maintenance of which may be favoured by artificial selection.

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STUDIES ON AN ANOPHTHALMIC STRAIN OF MICE. IV. A SECOND MAJOR GENE FOR ANOPHTHALMIA

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INTRODUCTION

INHERITANCE of eyelessness in mice has been discussed in a previous paper (CHASE 1942b) in which the method of analysis was the crossing of the inbred eyeless strain with other strains. The present study considers another method suggested at that time, involving a control of modifiers. This was made possible by a reverse mutation toward normal eyes in the eyeless strain. From the mutant individual two sublines have been developed, one with 98 percent anophthalmic mice, the other with 96 percent normals (preliminary report CHASE 1942a).

The fundamental phenomenon in the embryology of the eyeless strain is the inhibition of the growth of the optic vesicle at ten days or earlier, just after the normal formation of the vesicle (CHASE and CHASE 1941). Because of variations in the degree of inhibition, ten percent of the original strain have "small" or "medium" eyes as observed at birth or postmortem.

From the previous study (CHASE 1942b) of crosses of anophthalmic strain B with strain C57 Black and with strain K, one major factor difference, *ey* recessive for eyelessness, was deduced. From results of crosses with strains L and H, two hypotheses were suggested. In one hypothesis two major factors were present with one dominant sufficient for normal eyes (double recessive necessary for eyelessness) and strong minus modifiers from strain B. In the other hypothesis there was one major factor with dominance of normal eyes and strong plus modifiers from L and H The present paper will give additional evidence that the original anophthalmic strain is homozygous for one major factor, *ey ey*, and will present data to show that it is homozygous for another major factor for anophthalmia, present also in C57 Black but not in L and H.

HISTORY OF MUTANT

The anophthalmic strain B has the genotype *CCAAbbddSS*. The original animals (from strain R) were obtained in 1938 from DR. C. C. LITTLE of the ROSCOE B. JACKSON MEMORIAL LABORATORY at Bar Harbor, Maine. LITTLE observed reduced eyes in offspring from a wide cross, and selected for this character in subsequent generations. Twenty-one successive generations (16 at Illinois) of brother-sister matings have now been made. Birth records with confirmatory postmortem examinations have been employed for any study involving this character. In this strain, 88.3 percent are completely eyeless, 2.7 percent have very small eyes (both eyes or very small on one side and eyeless on the other), and 9.0 percent have intermediate eyes (for instance small left and eyeless right or small on both sides, etc.). Only a very small number of those with intermediate eyes have eyes large enough to be seen externally in the

adult. No normal eyes occur. The relatively few animals having one or both eyes of the very small category are combined with the completely eyeless animals as they were in the previous paper on the inheritance of eyelessness. Justification for this procedure is found in the embryological observations and in the breeding results from crosses with other strains where very small and eyeless behave similarly and not like small and medium.

In the eleventh generation of successive brother-sister matings (sixth at Illinois) a female appeared with a normal left eye and medium right eye, a condition much nearer normality than was every recorded in this strain. No fault in the record is possible because this female had the typical dilute brown agouti color characteristic of the strain, and crosses with any other animals present in the colony at that time would have given a different color. This female, mated with a completely eyeless brother, produced offspring indicating that she was heterozygous for a factor for normal eyes. From this mating two sublines have been selected, one with 98 percent anophthalmics and the other with 96 percent normals.

The two sublines derived from the mutant female (normal left and medium right) crossed with her eyeless brother are named Be_1 for the 98 percent eyeless group and Be_2 for the 96 percent normal group. By successive brother-sister matings Be_1 has reached the seventh generation and Be_2 has reached the fifteenth generation beyond the original mutant. After the third generation these two selected sublines have remained constant in expression. The rapidity of fixation indicates that at most very few inherited modifiers were heterozygous at the time selection for the two sublines started.

Line B was continued through the eyeless sister of the mutant and the same eyeless male.

CROSSES WITH OTHER STRAINS

The development of the two sublines is presented in table 1, and also the results of crosses of Be_2 (normal subline) with other strains are indicated. Since no significant differences were found between males and females, the sexes are combined in this paper.

In the crosses involving C57 Black, the subsequent F_2, and backcrosses to Be_2, it appears that Be_2 and C57 Black do not possess the same alleles for normal eyes and that modifiers alter the ratios. More information is gathered from the two crosses presented at the bottom of table 1. The original mutant female crossed with a normal male which was an F_1 individual from strain B by C57 Black (therefore *Ey ey* in genotype) produced 63.7 percent normal offspring. A similar reciprocal cross of an F_1(B×C57 Blk) female by a son of the original mutant produced 62.8 percent normal offspring. This evidence suggests either a new intermediate allele or, more probably, a new independent factor for normal eyes not showing dominance.

All offspring from the series of crosses involving Be_2 with strains L and H are normal. When this result is compared with the segregating results of similar crosses involving B with L and H (CHASE 1942b), it strengthens the suggestion of a new independent factor for normal eyes rather than a new

TABLE 1

Percentage of animals with normal eyes, intermediate eyes, and no eyes. L=left eye, R=right eye, norm=normal, med=medium, eyl=eyeless, inter=intermediate.

	PERCENTAGE			TOTAL NUMBER
	NORMAL	INTER-MEDIATE	EYELESS	
Strain B		9.0	91.0	1054
Strain Be				
Mutant norm L med R ♀×eyl ♂	7.7	38.5	53.8	39
F_2, norm L med R ♀×norm ♂	17.8	35.6	46.6	45
F_3, norm L med R ♀×norm ♂	83.3	16.7		30
F_3, norm ♀×norm ♂	87.0	8.7	4.3	23
F_2, eyl ♀×small L and R ♂		43.2	56.8	102
F_3, eyl ♀×eyl ♂		10.0	90.0	50
Be_1, F_4–F_8, eyl ♀×eyl ♂		2.3	97.7	343
Be_2, F_4–F_{16}, norm ♀×norm ♂	96.6	3.3		447
Be_2, F_6, inter ♀×norm ♂	100.0			28
Strain C57 Black	95.7	4.3	0.1	2200
Be_2×C57 Black	100.0			43
F_2	89.8	7.8	2.3	128
Backcross to Be_2	85.2	11.6	3.1	129
Second backcross to Be_2	78.3	21.7		23
Strain L	100.0			>1500
Be_2×L	100.0			22
F_2	100.0			99
Backcross to Be_2	100.0			19
Strain H	100.0			>1500
Be_2×H	100.0			32
F_2	100.0			85
Backcross to Be_2	100.0			42
Second backcross to Be_2	100.0			4
Mutant ♀×F_1 (B×C57 Blk) norm ♂	63.7	27.3	9.0	22
F_1 (B×C57 Blk) ♀×norm F_2 Be ♂	62.8	37.2		43

allele of *ey*. This normal subline derived from the mutant therefore makes it possible to decide between the two hypotheses advanced in the previous paper to explain the difference in results of crosses between anophthalmic strain B and normal strains L and H compared with crosses with C57 Black. Two major genes for normal eyes are present in L and H and only one of them in C57 Black.

CROSSES BETWEEN ANOPHTHALMIC SUBLINE AND NORMAL SUBLINE

Crosses between the Be_1 anophthalmic subline and the original B anophthalmic strain give results which agree reasonably with the regular B frequency of approximately 90 percent eyeless (table 1). Because of this, any great differ-

ence of modifiers between these strains may be eliminated from consideration. As the two inbred sublines, Be_1 (98 percent anophthalmic) and Be_2 (96 percent normal), are derived from the original mutant female of the eleventh generation of successive brother-sister matings, they offer a method of analysis of inheritance wherein the modifiers must be nearly the same and the major factor to be studied represents the only difference. Eyeless by eyeless matings in the F_2 and F_3 of these subline crosses do not all give approximately 98 percent eyeless (table 2). This fact must be due to the fact that eyeless phenotypes do not always correspond with genotypes homozygous for both alleles for anophthalmia.

TABLE 2

Crosses between the two sublines, Be_1 anophthalmic and Be_2 normal. Eyl = eyeless, inter = intermediate, norm = normal, BX = backcross, ditto = same animal.

CROSS	♀	♂	PERCENTAGE			TOTAL NUMBER
			NORMAL	INTER-MEDIATE	EYELESS	
F_1	eyl	norm	14.8	72.2	13.0	54
BX	eyl	"	78.6	21.4		14
F_2	norm	eyl	17.4	60.9	21.7	23
F_2	eyl	"	9.1	81.8	9.1	11
F_3	norm	eyl	9.8	46.3	43.9	41
F_3	eyl	"		34.6	65.4	26
F_1	norm	eyl	52.6	47.4		38
BX	norm	"	5.0	40.0	55.0	20
BX	inter	"		29.4	70.6	17
F_2	inter	inter	30.0	47.5	22.5	40
F_2	inter	"	28.6	39.3	32.1	56
F_3	norm	eyl	15.3	83.0	1.7	59
F_3	eyl	"	2.1	33.3	64.6	48

No dominance is found for normal or eyeless when these two sublines are crossed. Furthermore, the heterozygote (table 2) has a complete range of expression from normal to eyeless and the two homozygotes both overlap into the intermediate group and possibly rarely with each other.

One non-hereditary modifier, a matroclinous tendency, was suspected early in this study. Normal mothers tend to shift the median of the range of expression toward normality in their offspring and eyeless mothers, toward eyelessness. Analyzed by "Student's" paired comparison method, the probability is .08, a border-line situation and suggestive only of a matroclinous tendency.

DISCUSSION

In CHASE (1942b) two hypotheses were suggested for the difference between the ratios of B by C57 Black crosses and of the B by L or H crosses. One hypothesis involved one major factor difference for both types of crosses and

greatly different modifiers to explain the ratio differences. The other hypothesis involved two major factors to explain the results of L and H crosses, as compared with C57 Black. By the latter hypothesis only one allele for normal eyes would be sufficient to produce normality. This is an example of duplicate factors (15:1).

Two inbred sublines, one anophthalmic and the other normal, were derived from the mutant female which occurred in the eleventh generation of successive brother-sister matings. Modifiers are thus controlled and an analysis of this new gene can be made. It shows no dominance, and the variability of expression is such that the heterozygote ranges from normal to eyeless, and both homozygotes overlap into the intermediate category. This lack of dominance of the second major factor and dominance of the first major factor agree extremely well with the B by L and H crosses (CHASE 1942b). For instance there were 88.7 percent normals in the F_2 generations (522 animals) rather than the 93.75 percent (15:1) to be expected with complete dominance of both major factors. Furthermore, C57 Black, having the normal allele of the first major gene for defective eyes, crossed with Be_2, having the normal allele of the second major gene, gives in the F_2 generation a percentage of 89.8 normals. This close agreement with the B by L and H F_2 is clear evidence that L and H have both major alleles for normality and B has the corresponding major alleles for anophthalmia.

The new gene appears from tests not to be an allele of the previously reported gene for anophthalmia but rather an independent factor. The original gene for anophthalmia *ey* must be named *ey-1* and the new mutant *Ey-2*. From crosses between various lines, the genotypes in relation to these genes for anophthalmia are:

Strain B, original anophthalmic	*ey-1 ey-1; ey-2 ey-2*
Subline Be_1, anophthalmic	*ey-1 ey-1; ey-2 ey-2*
Subline Be_2, normal	*ey-1 ey-1; Ey-2 Ey-2*
Strain C57 Black, normal	*Ey-1 Ey-1; ey-2 ey-2*
Strains L and H, normal	*Ey-1 Ey-1; Ey-2 Ey-2*

Of general interest for the field of genetics is the observation that eyelessness depends on the cumulative effect of two mutant alleles. When animals of the eyeless strain are crossed with normal strains L and H, there is in the F_2 a 15:1 ratio. This ratio, characteristic of duplicate factors, is very unusual among animals. Also of general interest is the occurrence of a reverse mutation in a mammal and the fact that it does not show dominance in the combinations tested, whereas *Ey-1* does show dominance.

SUMMARY

Ey-1 shows dominance over *ey-1*, the gene originally described for anophthalmia.

A mutant female occurred in the original anophthalmic strain. This female had a normal left and a medium right eye, far beyond the usual range of the anophthalmic strain.

Two sublines were developed from the mutant, an anophthalmic subline and a normal subline.

Ey-2, the new mutant gene, shows no dominance over *ey-2*, and the heterozygote ranges from normal to eyeless.

Normal eyes may occur in the presence of either *Ey-1* or *Ey-2*.

The allele *Ey-2* is homozygous in strains L and H but absent (*ey-2 ey-2*) in C57 Black, whereas *Ey-1* is homozygous in all three strains.

Both separate alleles for anophthalmia are present in strain B and in the anophthalmic subline (*ey-1 ey-1; ey-2 ey-2*).

The *Ey-2* allele for normal eyes and the *ey-1* for eyelessness are present in the normal subline.

This case seems to be one of the few examples of duplicate factors (15:1 ratio) found in animals.

The mutant allele *Ey-2* is a reverse mutation, an occurrence probably rare in mammals.

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GENETICS OF NATURAL POPULATIONS. XI. MANIFESTATION OF GENETIC VARIANTS IN DROSOPHILA PSEUDO-OBSCURA IN DIFFERENT ENVIRONMENTS

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INTRODUCTION

NATURAL populations of *Drosophila pseudoobscura* contain a great store of concealed recessive autosomal genes or gene complexes which, when homozygous, modify the viability, development rate, fertility, and other characters of their carriers. Data on the frequencies of different types of concealed variants have been published (DOBZHANSKY, HOLZ, and SPASSKY 1942 and previous work). The present article reports an attempt to compare the manifestations of some of these variants in different environments that can be created in laboratory experiments, and with different genetic residues. The survival value of a genetic variant is a function of its behavior in different environments which a species may encounter; some knowledge of the "norms of reaction" of the concealed variants is needed for an evaluation of the possible role of the concealed genetic variability in evolution.

We are indebted to the CARNEGIE INSTITUTION OF WASHINGTON for the support of our work, and to MISS A. M. HOLZ and MRS. N. P. DOBZHANSKY for assistance in making some of the calculations.

MATERIAL AND METHOD

An analysis of 326 second and 352 fourth chromosomes of *Drosophila pseudoobscura* from several localities in California was made by DOBZHANSKY, HOLZ, and SPASSKY (1942). From these samples 26 second and 22 fourth chromosomes were selected for the present investigation. The methods of analysis were described in the paper just referred to and need not be repeated here. The wild second chromosomes are preserved in stock by balancing them against a laboratory second chromosome which contains an inversion and the mutant genes upturned, bithorax, Bare, and glass (wild/*upt bx Ba gl* inversion). A fourth chromosome with an inversion and the mutant genes incomplete, hooked, and Curly is used to keep strains of wild fourth chromosomes in balanced condition (wild/*inc hk Cy* inversion). The 48 chromosomes selected by us do not represent a random sample of chromosomes found in natural populations. We have chosen chromosomes which, from previous work, were known to produce some, although not too drastic, departures from normal viability or normal development rate. It must also be noted that these chromosomes were kept in the balanced condition for from half a year to a year and a half prior to the start of the present work; some variants not originally present in these chromosomes might have arisen by mutation during this time interval.

The main body of the data consists of observations on the performance of homozygotes for various second or fourth chromosomes at three temperatures

(25½°, 21°, and 16½°C, the last fluctuating from 15° to 17° about thrice within each hour). In the experiments on second chromosomes, 12 ♀♀ and 12 ♂♂ of the constitution wild/*up bx Ba gl* inversion from a given strain are placed in vials with food, and left there for three days to mature. The flies are then transferred 30 times to half-pint milk bottles with cornmeal-molasses-agar medium at daily intervals. Oviposition takes place at room temperature, but the bottles with the eggs are placed in incubators or in the cold room at the temperatures indicated above; the first bottle is placed at 16½°, the second at 21°, the third at 25½°, the fourth again at 16½°, etc. Therefore, ten bottles with the offspring of the same group of parents develop at each temperature. Since the fecundity of the flies varies from day to day and with age, the variable of population density is introduced; the number of parents per culture is adjusted to produce overpopulation of some and underpopulation of other cultures. The strains with wild fourth chromosomes are treated like those with the wild seconds, except that, owing to the diminished fecundity of Curly flies, 15 or 16 parents of each sex are placed in each culture. The flies hatching in the cultures are counted daily. In calculations, the occasional Bare homozygotes are ignored and the few crossovers are added to the Bare or Curly classes respectively.

CONTROL EXPERIMENTS ON VIABILITY

Normal viability, normal development rate, etc., are defined as those possessed by flies which carry two second or two fourth chromosomes taken at random from a natural population. Viability and development rate of the homozygotes for given wild second or wild fourth chromosomes are measured by observing the segregation ratios of wild type versus Bare or Curly flies which appear in the experimental cultures. In other words, the wild type homozygotes are compared with the wild/Bare or wild/Curly heterozygotes. To translate the results in terms of the normal viability or development rate, the viability and development rate of the Bare and Curly heterozygotes must be known. This was accomplished by intercrossing wild/Bare (or wild/Curly) heterozygotes from *different* cultures and observing the segregation ratios in the offspring (DOBZHANSKY, HOLZ, and SPASSKY 1942). These experiments were done at room temperature (20°–24°C). Since it cannot be taken for granted that the performances of Bare and Curly are similar at other temperatures, we were forced to make new control experiments for the present investigation.

Strains carrying different wild second chromosomes were intercrossed in various combinations; similar intercrosses were made between strains with different wild fourth chromosomes. Ten "series" were made for the second chromosomes; by a "series" is meant a combination of two given strains. Six cultures of each series were kept at each of the three temperatures: 16½°, 21°, 25½°C. Thus, 180 cultures in all were raised; 35,024 flies hatched in them, 12,109, or 34.573±0.252 percent, of which were wild type. The controls of DOBZHANSKY, HOLZ, and SPASSKY (1942) contained 23,873 flies 33.196±0.305

percent of which were wild type. Combining all the data, we obtain the figure 58,897 flies, 34.015±0.200 percent of which were wild type. Compared to the ideal ratio, 33.333 percent, this is a small but statistically significant deviation; the viability of Bare heterozygotes is very slightly below normal. Very important for us is the behavior of the separate series of the intercrosses at different temperatures. Examination of the variance among the 180 cultures gives the following result:

	χ^2	df	P
Cultures	156.445	150	0.70
Temperatures	17.762	20	0.70–0.50
Series	10.925	9	0.30–0.20

No heterogeneity is detected. Within our experiments, the relative viability of Bare heterozygotes is independent of the intercross made, of temperature, and of the accidental differences between cultures. The possibility that the viability of Bare may be influenced by the population density in the cultures was examined by constructing a correlation table between the total numbers of flies hatched in each culture (which varied from fewer than 50 to nearly 500) and the percentage frequency of wild type in that culture. The result is negative (correlation coefficient +0.1175±0.0735). We may conclude that the viability of Bare is very nearly normal at the conditions encountered in our experiments.

Curly behaves differently from Bare. Ten series of crosses were raised, with ten cultures at each of the three temperatures. Out of the 300 cultures, 281 produced offspring; 31.373±0.212 percent of the 48,165 flies obtained were wild type. Adding the 29,081 flies raised in the control experiment of DOBZHANSKY, HOLZ, and SPASSKY, the grand total of 77,246 flies is obtained, of which 31.919±0.168 percent are wild type. Taken at face value, this figure would suggest that Curly heterozygotes have a slightly better than normal viability. However, experimental errors are almost certainly involved here: in some cultures Curly comes close to overlapping wild type, and some wild type flies with wavy wing might be occasionally misclassified as Curly. Examination of the variances shows the following:

	χ^2	df	P
Cultures	361.775	251	<0.01
Temperatures	35.457	20	0.02
Series	43.479	9	<0.01

The material is quite heterogeneous. The heterogeneity between the series, however, is due mostly to one of them—namely, the cross Andreas 1035 ×Andreas 1212—giving aberrant results (28.70 percent of wild type in 6000 flies). The heterogeneity between the temperatures is not great and shows no regularity: in some series the cultures raised at the lowest, and in other series those raised at the intermediate or at the highest temperatures produce most wild type. But the heterogeneity between cultures within a series and a temperature is appreciable; the viability of Curly is evidently influenced by

TABLE 1

Total numbers of the flies (n) and percentages of wild type among them (%) in the control experiments carried at different temperatures.

	DAY OF HATCHING	16½°C		21°C		25½°C	
		n	%	n	%	n	%
Second Chromosome	1	1,327	37.53	1,165	34.94	1,278	35.45
	2	2,100	35.95	2,856	35.61	2,531	37.42
	3	2,200	33.36	2,486	34.11	2,372	32.97
	4	1,968	37.45	1,796	35.80	1,572	32.25
	5	1,746	34.42	1,189	34.82	910	35.05
	6	1,345	33.90	799	33.04	549	34.24
	7	949	33.93	523	31.55	385	32.47
	8	601	32.61	299	31.44	190	30.00
	9	462	31.39	110	39.09	87	36.78
	10	375	29.87	41	29.27	76	47.37
	11	218	27.06	19	21.05	52	34.62
	12	176	29.55	—	—	38	34.21
	13	88	29.55	—	—	15	33.33
	14	77	11.69	—	—	5	80.00
	15	28	21.43	—	—	—	—
	Total	13,660	34.47	11,283	34.66	10,060	34.65
Fourth Chromosome	1	1,362	39.21	2,078	39.32	2,477	42.47
	2	2,090	36.84	3,100	34.19	3,695	33.18
	3	2,437	32.46	2,314	32.50	2,733	29.27
	4	1,667	30.95	2,188	29.43	1,986	28.95
	5	1,545	31.59	1,683	29.06	1,531	28.74
	6	1,408	31.11	1,454	25.72	1,056	28.22
	7	1,304	29.83	1,293	28.23	869	30.38
	8	1,056	25.95	797	26.35	595	29.92
	9	866	25.64	525	24.00	422	28.67
	10	520	21.73	378	26.19	255	27.84
	11	452	24.34	235	25.53	163	24.54
	12	312	21.47	194	28.35	—	—
	13	211	23.70	51	13.73	—	—
	14	160	18.75	—	—	—	—
	15	125	24.80	—	—	—	—
	Total	15,515	31.09	16,290	31.05	15,782	32.09

some uncontrolled factors. Population density is not one of these factors: correlation between density and percentage of the wild type class is -0.050 ± 0.060, which is not significant. Curly is not as good a viability standard as Bare is.

CONTROL EXPERIMENTS ON THE DEVELOPMENT RATE

The intercrosses made for the comparison of the viability of Bare and Curly respectively with that of wild type may be used also to study the development

rates of these mutants. If Bare or Curly flies develop more slowly than wild type ones, the proportion of wild type will evidently be greater among the flies hatching early than among those hatching late in the cultures; if Bare or Curly accelerate the development, the proportion of wild type will increase from the early to the late counts. The data are summarized in table 1; the cultures in which all the flies have hatched in a single day are ignored, which explains the fact that the totals shown in table 1 do not add up to those quoted above.

In the experiment on the second chromosome, the frequencies of wild type and Bare show no systematic change from the early to the late counts at 25½°C; a test of homogeneity gives $\chi^2 = 16.74$, which, for 12 degrees of freedom (the 13th and 14th days combined), has the probability of occurring by chance between 0.20 and 0.10. No heterogeneity is observed at 21° ($\chi^2 = 6.59$, df = 10, P = 0.80–0.70). But at 16½° the wild type class is more frequent in the early than in the late counts ($\chi^2 = 30.62$, df = 14, P < 0.01). Bare flies develop more slowly than wild type at 16½°, but there is no difference at 21° or at 25½°.

The experiments on the fourth chromosome (table 1) show that the proportion of wild type is significantly higher and that of Curly significantly lower in the early than in the late counts at all three temperatures. The development of Curly is slower than that of wild type. It is difficult to tell whether this slowing down is relatively greater at the higher or at the lower temperatures; no striking differences are apparent.

VIABILITY OF SECOND CHROMOSOME HOMOZYGOTES

The data on the viability of homozygotes for different wild second chromosomes at different temperatures are summarized in table 2, which shows the total numbers of flies which hatched in all cultures raised at a given temperature (n) and the percentage of wild type flies (%) among them. In this and the following tables, the individual chromosomes are designated by the abbreviated name of the collecting station from which they came and by a number (AA—Andreas A, PA—Pinon Flats A, KA and KD—Keen A and Keen D respectively, M—Mission Canyon, see WRIGHT, DOBZHANSKY, and HOVANITZ 1942). The column P indicates the statistical probability of such differences being due to chance.

It is evident that the viability of the homozygotes for some chromosomes varies greatly with temperature, while that of others is rather constant within the temperature range tried. Chromosome AA 1015 displays the most striking sensitivity to temperature: at 16½° the homozygotes for this chromosome possess a viability only slightly inferior to normal, at 21° the chromosome acts as a semilethal, and at 25½° as a complete lethal. Chromosome KD 745 gives homozygotes which seem to possess a viability superior to normal at 16½°, a viability definitely below normal at 21°, and semilethal at 25½°. In all, 15 out of the 26 chromosomes show a significant change in viability effects with temperature. Homozygotes for 11 of them survive best at the lowest temperature and deteriorate in viability as the temperature increases. Three chromosomes (AA 958, AA 1003, and PA 858) are at their best at the intermediate

TABLE 2

Second chromosomes. Total numbers of the flies (n), percentages of wild type among them (%), and the statistical significance of the observed differences in the outcome of the experiments at different temperatures.

CHROMOSOME	16½°C		21°C		25½°C		χ^2	P
	n	%	n	%	n	%		
AA 958	568	0.00	535	13.08	398	1.01	120.8	<0.01
AA 966	711	26.02	326	12.58	381	2.36	104.8	<0.01
AA 974	1769	32.45	1482	34.68	1365	30.84	4.9	0.10–0.05
AA 975	558	35.84	543	35.73	469	32.20	1.9	0.50–0.30
AA 995	1869	31.67	1386	27.20	1085	22.67	28.5	<0.01
AA 1003	1007	31.58	1050	34.57	828	26.93	12.4	<0.01
AA 1015	1033	29.71	695	14.24	459	0.00	197.5	<0.01
AA 1023	855	32.28	1003	35.29	1028	32.68	2.3	0.50–0.30
AA 1029	2187	33.10	1998	32.68	2024	32.95	0.1	0.98–0.95
AA 1093	2350	32.72	2151	34.45	2066	32.43	2.3	0.50–0.30
AA 1178	694	35.16	867	34.83	811	30.70	4.5	0.20–0.10
AA 1179	569	33.92	637	35.48	426	32.86	0.9	0.70–0.50
PA 736	791	27.81	763	18.35	713	8.98	87.6	<0.01
PA 748	913	23.00	750	13.60	766	9.92	57.2	<0.01
PA 784	1020	26.86	891	21.44	796	20.48	12.5	<0.01
PA 798	1340	30.00	1281	34.19	967	34.54	7.2	0.05–0.02
PA 823	1148	28.92	975	29.64	1070	30.65	0.8	0.70–0.50
PA 832	716	33.24	492	37.20	542	33.76	2.2	0.50–0.30
PA 841	1029	32.17	919	26.66	477	11.53	72.1	<0.01
PA 858	1276	31.50	1151	37.01	959	30.03	13.5	<0.01
PA 863	1911	36.42	1728	36.40	1549	30.60	16.2	<0.01
KA 667	1295	36.06	1320	33.56	985	21.62	59.8	<0.01
KD 745	1378	35.20	1081	24.42	685	9.20	163.1	<0.01
KD 775	1688	27.13	1635	27.83	1367	22.53	12.5	0.01
KD 787	1567	29.36	1333	30.53	951	33.44	4.7	0.10–0.05
M 3	1084	31.64	970	33.40	1024	30.47	2.0	0.50–0.30

temperature (21°) and decline at the higher as well as at the lower temperatures. PA 858 is especially interesting because its viability at 21° is almost significantly above normal (37.0 ± 1.42 percent, instead of the normal 33.3 to 34.0 percent); it gave a superior to normal record also in the experiments of DOBZHANSKY, HOLZ, and SPASSKY (1942) carried at room temperature; yet, at 16½° and 25½° it seems to have a subnormal viability. No chromosomes give homozygotes with an optimal viability at the highest temperature tried (25½°); KD 787 approaches nearest to that condition, but the variations observed are not quite statistically significant. PA 798 is equally viable at 21° and 25½°, and less viable at 16½°. The relatively greatest variations in the viability with temperature tend to occur in chromosomes which deviate from normal throughout the temperature range studied, and chromosomes which produce normally viable homozygotes at any one temperature are relatively stable. Chromosome AA 1015, however, is an exception to this rule.

In the experiments of DOBZHANSKY, HOLZ and SPASSKY (1942) which were carried at room temperature, the chromosomes AA 975, AA 1003, AA 1023, PA 832, PA 858, PA 863, KA 667, and M 3 gave viability records superior to normal. In the present experiments, with the exception of KA 667 and M_3, these chromosomes give frequencies of the wild type class in excess of normal at the intermediate temperature, 21° (table 2). Although these positive deviations from the normal viability are mostly not quite significant statistically, the agreement between the different experiments makes them more trust-

TABLE 3

Second chromosomes. Tests of heterogeneity among the cultures.

CHROMOSOME	t°	χ^2	df	P	CHROMOSOME	t°	χ^2	df	P
AA 958	21	19.1	7	<0.01		16½	6.7	9	0.70–0.50
AA 966	16½	8.7	8	0.50–0.30	PA 784	21	34.9	9	<0.01
	21	10.0	4	0.05–0.02		25½	31.4	8	<0.01
	16½	6.2	9	0.80–0.70	PA 798	16½	13.1	9	0.20–0.10
AA 974	21	28.6	9	<0.01		21+25	15.6	18	0.70–0.50
	25½	7.8	9	0.70–0.50	PA 823	all	41.3	27	0.05–0.02
AA 975	all	36.7	25	0.10–0.05	PA 832	all	24.4	22	0.50–0.30
	16½	6.6	9	0.70–0.50		16½	20.8	9	0.02–0.01
AA 995	21	5.1	7	0.70–0.50	PA 841	21	14.6	9	0.20–0.10
	25½	22.2	9	<0.01		25½	10.7	7	0.20–0.10
	16½	18.2	9	0.05–0.02		16½	13.3	9	0.20–0.10
AA 1003	21	14.4	9	0.20–0.10	PA 858	21	11.2	9	0.30–0.20
	25½	4.2	9	0.90–0.80		25½	17.8	9	0.05–0.02
AA 1015	16½	14.1	8	0.10–0.05		16½	5.5	9	0.80–0.70
	21	25.3	7	<0.01	PA 863	21	9.9	9	0.50–0.30
AA 1023	all	21.8	29	0.90–0.80		25½	9.9	9	0.50–0.30
AA 1029	all	27.8	29	0.70–0.50		16½	22.6	9	<0.01
AA 1093	all	36.8	29	0.20–0.10	KA 667	21	10.4	9	0.50–0.30
AA 1178	all	28.4	19	0.10–0.05		25½	20.2	9	0.02–0.01
AA 1179	all	27.6	24	0.30–0.20		16½	12.5	9	0.20–0.10
	16½	14.5	9	0.20–0.10	KD 745	21	24.5	9	<0.01
PA 736	21	26.4	9	<0.01		25½	19.3	6	<0.01
	25½	31.9	9	<0.01		16½	18.7	9	0.05–0.02
	16½	24.8	9	<0.01	KD 775	21	19.3	9	0.05–0.02
PA 748	21	33.3	8	<0.01		25½	7.7	9	0.70–0.50
	25½	49.3	9	<0.01	KD 787	all	38.2	29	0.20–0.10
					M 3	all	60.6	29	<0.01

worthy. It is important, however, that not a single chromosome gave viability records superior to normal at all the temperatures. Herein probably lies the solution of the apparent paradox: the presence in natural populations of some chromosomes which give homozygotes with a higher than normal viability. The superiority of these chromosomes is confined to only a narrow range of environmental conditions, while in other environments which the species encounters in nature these chromosomes are deleterious (*cf.* DOBZHANSKY, HOLZ, and SPASSKY 1942).

As stated in the description of the experimental procedure, ten cultures were raised containing the offspring of the same group of parents at each of the three temperatures and for each chromosome. It is obvious that environmental conditions in different cultures grown at the same temperature are not uniform, and examination of the data shows that different cultures of the same series sometimes produce different proportions of wild type flies. An analysis of variance was made in the entire material; the results are summarized in table 3. Only a general χ^2 for all the cultures regardless of temperature was computed for those chromosomes which show no variations in the survival rate at different temperatures; whenever temperature effects were observed, the calculations were made for each temperature separately. Lethals, semilethals, and cultures in which the expected numbers of wild type individuals were less than five were disregarded.

Table 3 shows that, as a rule, no significant heterogeneity between cultures is observed for those chromosomes which give similar survival rates at different temperatures; chromosomes PA 823 and M3 are exceptions: they are not temperature sensitive but show significant heterogeneity among cultures. Temperature-sensitive chromosomes may or may not be sensitive to the diversity of conditions found among cultures at the same temperature. Thus, PA 798 and PA 863 are appreciably sensitive to temperature variations but not to other culture conditions. In contrast to this, PA 748 gives quite different frequencies of the wild type class in different cultures (16–41 percent at 16½°, 7–17 percent at 21°, and 0–24 percent at 25½°). Chromosomes AA 966, AA 995, AA 1015, PA 736, PA 784, PA 858, and KA 745 are sensitive to variations in culture conditions chiefly or only at those temperatures at which their viability is lowest; the reverse relation is observed for only a single chromosome—PA 841.

The only variable among the "culture conditions" which can be roughly measured is the population density. Tests were made to examine the effects of this variable. The cultures were broken up into classes according to the total numbers of the flies produced in them, frequencies of the wild type were computed for each class, and χ^2 was used to test the significance of the differences observed. In some cases the population density was responsible for at least a part of the heterogeneity observed among the cultures. Thus, in PA 736 the cultures with fewer than 50, with 50 to 100, and with 100 to 150 flies gave the following percentages of wild type:

t°C	FLIES PER CULTURE			χ^2	P
	<50	50–100	100–150		
16½	34.8	27.9	27.8	0.32	0.90–0.80
21	22.5	25.6	14.7	11.41	<0.01
25½	14.3	12.0	5.2	13.39	<0.01

At 25½° the homozygotes survive better in cultures with few flies than in

crowded cultures; at 21° there is either an optimum at a density of 50–100 flies per culture or else the deleterious effect of crowding begins to appear only in cultures with more than 100 flies; at 16½ the population density seems to have no effect. Deleterious effects of crowding are established also for chromosomes PA 841 at 16½° and for PA 858 at 25½°. On the other hand, homozygotes for some chromosomes survive relatively better in crowded cultures, as shown by the following example of PA 748:

t°C	FLIES PER CULTURE				χ^2	P
	<50	50–100	100–150	150		
16½	19.2	22.4	24.8	21.5	1.32	0.80–0.70
21	13.5	9.2	18.6	—	10.91	<0.01
25½	—	5.9	22.0	—	31.59	<0.01

Chromosomes KA 667 at 16½° and 25½°, AA 995 at 25½°, and AA 1015 at 21° are further examples of the type of behavior just shown for PA 748. All other chromosomes listed in table 3 as giving significant heterogeneities among cultures do not seem to differ from Bare in their sensitivity to the effects of crowding, at least not within the limits found in our experiments. The heterogeneity is due to some uncontrolled variables. In this connection it should be recalled that in the control experiment on the second chromosome no heterogeneity between cultures, due either to temperature, to crowding, or to other variables, was observed. The sensitivity to these environmental agents, therefore, is a special property of the reaction norms of some, but not of all, wild chromosomes.

VIABILITY OF FOURTH CHROMOSOME HOMOZYGOTES

The behavior of the fourth chromosomes is analogous to that of the seconds. Table 4 summarizes the data. Only six out of the 23 chromosomes studied show no significant alterations in the viability of homozygotes at different temperatures; the incidence of non-sensitive chromosomes observed here seems smaller than that found among the seconds, but this may be due to the fourth chromosomes having been tested more thoroughly (more flies raised per chromosome). A striking deterioration of the viability with increase in temperature is observed in AA 1035: the homozygotes for this chromosome show an almost normal viability at 16½°, a low viability at 21°, and are semilethal at 25½°. A similar but less striking change is observed in AA 1022, AA 1194, and PA 998. A lowering of the viability at 25½° with the outcomes at 21° and 16½° being alike is observed in AA 955 and AA 956. A preference for the lowest temperature with the two higher ones being approximately equivalent is found in AA 1105, PA 734, PA 988, and KA 688. An optimum at the intermediate temperature is recorded in AA 1052. An apparent preference for the highest, or for the highest and the lowest, temperature is suggested for AA 981, AA 1005, AA 1042 PA 727, PA 851, and KA 574. This last type of behavior has

TABLE 4

Fourth chromosomes. Total numbers of the flies (n), percentages of wild type among them (%), and the statistical significance of the observed differences in the outcome of the experiments at different temperatures.

CHROMOSOME	16½°		21°		25½°		x^2	P
	n	%	n	%	n	%		
AA 955	3600	31.86	2559	32.79	1686	17.62	137.9	<0.01
AA 956	1131	28.74	1379	27.48	1058	21.08	19.3	<0.01
AA 981	1862	2.42	1772	1.98	1549	4.26	17.42	<0.01
AA 1005	2384	0.29	2184	0.69	2237	1.21	13.6	<0.01
AA 1022	3455	30.01	3152	23.70	2426	18.01	112.8	<0.01
AA 1042	2464	13.92	2311	10.60	2102	12.37	12.1	<0.01
AA 1052	2965	23.31	2692	28.23	2823	23.52	22.9	<0.01
AA 1094	1637	31.70	1818	31.79	1972	29.06	4.3	0.20–0.10
AA 1105	3511	31.30	2857	23.70	3205	23.40	69.0	<0.01
AA 1194	2829	29.87	2329	26.32	2265	17.57	104.6	<0.01
AA 1212	1484	8.63	1615	8.54	1095	7.03	2.6	0.30–0.20
AA 1035	2621	30.29	2514	23.23	1808	10.23	248.0	<0.01
PA 727	2511	29.27	1751	25.19	2043	29.32	10.5	<0.01
PA 734	2340	31.24	1642	27.77	1887	28.25	7.1	0.05–0.02
PA 789	3050	15.02	2427	15.37	2685	14.53	0.7	0.70–0.50
PA 851	2253	30.54	1529	18.97	1594	26.35	63.5	<0.01
PA 988	3588	31.61	2935	25.62	3110	28.20	28.7	<0.01
PA 998	2166	23.59	1693	19.43	1705	16.83	28.0	<0.01
PA 1004	2895	31.54	2098	29.27	2279	30.01	3.2	0.30–0.20
KA 574	1473	0.68	1109	0.72	895	3.24	32.3	<0.01
KA 688	2674	32.20	2605	26.83	2123	26.85	23.9	<0.01
KD 731	1830	14.26	1335	11.91	1280	12.11	4.9	0.10–0.05
M 3	1415	30.60	1066	31.89	1412	34.14	4.1	0.20–0.10

not been recorded among second chromosomes, but it may be noted that only in PA 851 are considerable changes observed.

Table 5 summarizes the results of the tests of heterogeneity among the cultures. The data are treated as described above for the second chromosomes. Among the 6 chromosomes which show no temperature sensitivity, only one (PA 1004) fails to display a significant heterogeneity among cultures. This may be in part a spurious phenomenon, since the control experiment established that our marker, the mutant gene Curly, is itself sensitive to culture conditions (see p. 272). However, the heterogeneity shown by homozygotes is so striking in at least some cases (AA 1212, PA 789, M 3) that it cannot be accounted for by the relatively slight irregularities in the behavior of Curly. Furthermore, no significant heterogeneity among cultures within a temperature series is observed in some chromosomes which are sensitive to temperature changes (AA 956, PA 727, PA 851, PA 988). Some chromosomes show significant heterogeneities both between and within each temperature series (AA 1042, AA 1052, AA 1105, PA 734, PA 998). Still others show no heterogeneity at low temperatures but do at higher ones, or *vice versa*. It is clear that as great a variety of

TABLE 5

Fourth chromosomes. Tests of heterogeneity among the cultures.

CHROMOSOME	t°	χ^2	df	P	CHROMOSOME	t°	χ^2	df	P
AA 955	16½	5.5	9	0.80–0.70	AA 1035	16½	11.7	9	0.30–0.20
	21	8.1	9	0.70–0.50		21	24.9	9	<0.01
	25½	93.2	9	<0.01		25½	43.0	9	<0.01
AA 956	16½	14.2	9	0.20–0.10	PA 727	16½	10.2	9	0.50–0.30
	21	11.3	9	0.30–0.20		21	14.2	9	0.20–0.10
	25½	15.2	8	0.10–0.05		25½	6.9	9	0.70–0.50
AA 981	16½	20.8	9	0.02–0.01	PA 734	16½	23.7	9	<0.01
	21	10.3	9	0.50–0.30		21	15.4	9	0.10–0.05
	25½	10.2	9	0.50–0.30		25½	18.3	9	0.05–0.02
AA 1022	16½	8.9	9	0.50–0.30	PA 789	all	539.4	29	<0.01
	21	11.9	9	0.30–0.20	PA 851	16½	6.7	9	0.70–0.50
	25½	18.3	9	0.05–0.02		21	8.5	8	0.50–0.30
AA 1042	16½	32.7	9	<0.01		25½	2.6	8	0.98–0.95
	21	26.2	9	<0.01	PA 988	16½	4.2	9	0.95–0.90
	25½	85.5	9	<0.01		21	12.0	9	0.30–0.20
AA 1052	16½	48.3	9	<0.01		25½	7.8	9	0.70–0.50
	21	21.8	9	0.02–0.01	PA 998	16½	24.2	9	<0.01
	25½	16.9	9	0.05–0.02		21	40.4	9	<0.01
AA 1094	all	53.0	28	<0.01		25½	52.2	9	<0.01
AA 1105	16½	20.6	9	0.02–0.01	PA 1004	all	31.2	28	0.50–0.30
	21 & 25½	44.9	18	<0.01	KA 688′	16½	12.3	9	0.20–0.10
AA 1194	16½	14.9	9	0.10–0.05		21	10.4	9	0.50–0.30
	21	34.8	9	<0.01		25½	17.9	9	0.05–0.02
	25½	45.0	9	<0.01	KD 731	all	49.9	29	<0.01
AA 1212	all	112.0	29	<0.01	M 3	all	111.5	26	<0.01

norms of reactions is observed among the fourth as among the second chromosomes.

Wherever significant heterogeneities between cultures were observed, tests were made for the possible effects of the population density. The chromosome PA 789 is an example of a better survival in less crowded cultures. The percentages of wild type are as follows (combined data for all temperatures):

FLIES PER CULTURE							χ^2	P
<100	100–150	150–200	200–250	250–300	300–350	350–400		
32.7	16.7	26.5	23.8	14.3	18.1	6.4	150.5	<0.01

The chromosomes AA 1035, AA 1105, and PA 734 behave more or less similarly to PA 789. On the other hand, in AA 955 the homozygotes survive relatively better in crowded cultures at 25½° (there is no appreciable effect of the population density at lower temperatures):

FLIES PER CULTURE			x^2	P
<100	100–200	200		
6.1	12.7	22.3	39.4	<0.01

AA 1052 and AA 1212 also survive relatively better at high population densities (at all three temperatures). In still other chromosomes an optimum at a certain intermediate population density is indicated. The percentages of wild type in PA 998 are given below as an example of this class:

t°	FLIES PER CULTURE						x^2	P
	<100	100–150	150–200	200–250	250–300	300		
16½	27.6	31.3	26.6	23.7	19.5	23.1	13.0	0.05–0.02
21	18.8	25.1	22.0	17.7	—	—	8.9	0.05–0.02
25½	5.7	12.4	17.1	26.8	17.2	—	31.4	<0.01

Finally, in AA 1042 there is a slight but significant increase in the survival rate at high densities at 16½°, no density effect at 21°, and a sharp reduction of the viability at high densities at 25½°. Thus, there is a remarkable diversity of responses to the population density variable among the wild fourth chromosomes.

DEVELOPMENT RATE AND VISIBLE MUTANTS

DOBZHANSKY, HOLZ, and SPASSKY (1942) found that between one-third and one-half of the wild second and fourth chromosomes cause the development of the homozygotes to be slower than normal, while some few chromosomes make the homozygotes develop faster than normal. The effects of a chromosome on the development rate are detected through comparison of the wild type: Bare (or Curly) ratios in the early and late counts in the test cultures (*cf.* table 7). The type which develops slowly is more frequent in the late than in the early counts. Among the 26 second chromosomes used in the present experiments, 12 chromosomes were classed, on the basis of the DOBZHANSKY, HOLZ, and SPASSKY data, as permitting the homozygotes to develop at the normal rate, nine as producing slow, and five very slow development. Similarly, among the 22 wild fourth chromosomes concerned in the present experiments, seven were classed as normal, nine as slow, and four as very slow. As shown above, the effects of some of these chromosomes on the viability of the homozygotes are modified very appreciably by temperature, by population density, and by other culture conditions. In contrast to this, environmental modifications of the relative development rates were not detected. If the homozygotes for a given chromosome develop as fast as or slower than their heterozygous sibs at one temperature or at a certain population density, they do so also at other temperatures and population densities tried. The only apparent exception from

this rule is the second chromosome PA 784. The homozygotes for this chromosome develop slightly but significantly slower than their heterozygous sibs at 16½° and at 25½°; the figures for 21°, if taken at face value, also suggest some delay in the hatching of the homozygotes, but a statistical test shows the difference to be below the level of significance (P between 0.5 and 0.3). Of course, failure to detect striking modifications of the relative development rates by the environmental factors does not exclude the possibility that relatively slight modifications do occur.

Some of the wild chromosomes contain recessive mutant genes which produce visible structural changes in homozygotes. The expression of these visible mutants is sometimes modified in different environments. The most interesting case of this sort found in the present experiments concerns the fourth chromosome AA 1194. Homozygotes for this chromosome usually show branched or extra veins in the cultures raised at 16½°; at 21° not all flies have extra veins, but another character makes its appearance in some individuals—namely, notched wing margins; at 25½° extra veins are very rare, but notched wings are present in many flies. Whether the extra veins and the notched margins are due to the same or to different genes located in the AA 1194 chromosome is not known. At 25½° the homozygotes for the second chromosome PA 841 have shortened wings (of the type observed in the small wing mutant of *Drosophila melanogaster*), and some individuals also have deformed legs; these characters are hardly noticeable at 21°, and not at all at 16½°. The second chromosome KD 787 makes short stout bristles at 21° and 25½°, while the bristles are more nearly normal at 16½°. The fourth chromosome AA 955 causes the homozygotes raised at 25½° to have very rough, frequently deformed eyes and sometimes also deformed legs; at 21° legs are normal and rough eyes show in most males but in only a few females; at 16½° the females are normal and only a few of the males have slightly roughened eyes. Bobbed-like changes in the bristles and the abdomen are observed in homozygotes for the fourth chromosome AA 1052, especially in the females and at 25½°, less at 21°, and very little at 16½°. Several other visible mutants had their expression not appreciably modified by temperature in these experiments.

INFLUENCE OF GENETIC MODIFIERS

Theoretically, the manifestation of a genetic character depends not only on the external environment but also on the internal "genetic" environment, the whole genotype of the organism. In the experiments reported above, the genetic environment was kept as far as possible constant, since the same tester strains (*upt, bx Ba gl* inversion and *inc hk Cy* inversion) were used to examine the effects of homozygosis for all wild second and all wild fourth chromosomes respectively.[1] The experiments now to be described were designed to study the effects of homozygosis for certain wild second and fourth chromosomes in de-

[1] Some variations in the genetic environment were present, since the method of analysis used (DOBZHANSKY, HOLZ, and SPASSKY 1942) permitted some chromosomes of the wild strains to slip undetected into the tester strains.

liberately varied genetic environments. For this purpose, five wild second and four wild fourth chromosomes were chosen. From the experiments with constant genetic environment the effects of these chromosomes in homozygous condition were known to be as follows (*cf.* tables 2–5).

Second chromosomes

AA 1003—viability normal at 16½° and at 21°, slightly inferior to normal at 25½°; development very slow; not sensitive to population density or to other culture conditions. AA 1015—viability almost normal at 16½°, semilethal at 21°, lethal at 25½°; development slow; high population density favorable. AA 1178—viability normal or above normal at 16½° and 21°, normal or below normal at 25½°; development normal; not sensitive to population density or culture conditions. KA 667—viability apparently above normal at 16½°, normal at 21°, definitely below normal at 25½°; development very slow; not sensitive to population density or to culture conditions. KD 745—viability normal or superior at 16½°, below normal at 21°, semilethal at 25½°; development slightly slower than normal; sensitive to culture conditions other than population density.

Fourth chromosomes

AA 955—viability normal at 16½° and 21°, close to semilethality at 25½°; development slow; not sensitive to population density or culture conditions. AA 1035—viability normal at 16½°, subnormal at 21°, semilethal at 25½°; development slightly slow; most viable at low population densities; sensitive to culture conditions. PA 851—viability normal or subnormal at 16½°, close to semilethality at 21°, and again subnormal at 25½°; development rate normal not sensitive to population density or to culture conditions. PA 998—viability subnormal and uniform at all the temperatures tried; development rate normal; sensitive to culture conditions other than population density.

All the above second and fourth chromosomes came from populations of Mount San Jacinto, California. Five wild strains coming from different parts of the distribution area of *Drosophila pseudoobscura* were chosen to provide a variety of genetic backgrounds on which to study the effects of the San Jacinto second and fourth chromosomes. These strains came from: (1) Willapa Bay, Washington, (2) Mount Campbell, Colorado, (3) Borego Valley, California, (4) Pachuca, Mexico, and (5) Quezaltenango, Guatemala. The experimental procedure was as follows. In six successive generations, males from the ***upt bx Ba gl*** inversion and ***inc hk Cy*** inversion strains were outcrossed to females from each of the five wild strains just enumerated; stocks with the ***upt bx Ba gl*** inversion and ***inc hk Cy*** inversion chromosomes with each of the five sets of "geographic" modifiers were thus obtained. Bare females from each stock were then outcrossed to males homozygous for each one of the five San Jacinto wild second chromosomes (a total of 25 crosses). In the next generation, Bare females and males were selected in each cross and inbred; in the progeny, non-Bare males were taken and crossed to Bare females from the stocks with the respective "geographic" modifiers; in the next generation, Bare

females and males were inbred, next non-Bare males outcrossed to Bare females from the "geographic" stocks, etc. The alternation of crossing and inbreeding was repeated four times. As a result, 25 strains were obtained, each containing a known San Jacinto second chromosome in opposition to the *upt bx Ba gl* inversion chromosome, with all or most other chromosomes derived from a known geographic strain. A similar procedure was followed for the fourth chromosomes. Each of the resulting 20 strains contains a known San Jacinto fourth chromosome in opposition to the *inc hk Cy* inversion fourth, with all or most other chromosomes from desired geographic strains. The usual tests were then made: 12 pairs of Bare (or 15 pairs of Curly) flies from each strain were kept for three days in vials with food, and then transferred ten times at daily intervals to bottles with the regular cornmeal-molasses-agar culture medium. All the bottles developed in an incubator at 21°. Daily counts of the hatching progenies were made as usual. The results are summarized in table 6.

TABLE 6

Influence of genetic modifiers. Total number of flies (n), percentages of wild type among them (%), and the statistical significance of the observed differences in the outcome of the experiments with different modifiers. II—second chromosomes, IV—fourth chromosomes.

CHROMOSOME	MODIFIERS										χ^2	P
	WILLAPA BAY		CAMPBELL		BOREGO		PACHUCA		QUEZALTE-NANGO			
	n	%	n	%	n	%	n	%	n	%		
AA 1003 (II)	2579	32.07	3188	33.91	2666	33.20	4062	28.72	1797	29.94	27.0	<0.01
AA 1015 (II)	2958	12.81	3637	31.15	3150	30.82	3121	24.73	727	24.54	362.2	<0.01
AA 1178 (II)	2155	34.99	3595	30.79	2181	33.56	2467	31.86	2136	32.12	12.6	0.02–0.01
KA 667 (II)	1305	33.05	2190	29.91	2178	32.87	1117	29.81	1424	33.43	11.2	0.05–0.02
KD 745 (II)	3857	33.63	4956	31.56	4211	32.68	2512	29.86	3017	32.32	11.3	0.05–0.02
AA 955 (IV)	3153	28.35	4561	31.83	4631	31.12	1846	30.50	1483	28.52	14.2	<0.01
AA 1035 (IV)	3364	27.07	3095	32.47	3209	23.37	2213	28.38	3028	29.39	66.9	<0.01
PA 851 (IV)	1695	25.00	1279	25.72	2607	32.99	2275	28.18	1331	24.49	46.8	<0.01
PA 998 (IV)	3590	29.34	3203	27.22	4252	28.27	2291	29.81	1753	25.33	13.9	<0.01

It is evident that the survival rates of homozygotes for wild second and fourth chromosomes may be influenced by modifying genes in other chromosomes. The χ^2 tests show significant or nearly significant differences in the proportions of wild type flies appearing in the cultures with different modifier systems for every chromosome. The degree of modification, however, is not equal in all cases. The second chromosomes AA 1003, AA 1178, KA 667, KD 745, and the fourth AA 955 produce homozygotes that have normal or slightly subnormal viabilities with every one of the five modifier systems tried; it is interesting to recall that KA 667 and KD 745 are very sensitive to temperature variations (*cf.* table 2). In contrast to this, homozygotes for the second chromosome AA 1015 have a viability only slightly below normal with the Borego Valley and Mount Campbell modifiers, a viability clearly below normal with the Pachuca and Quezaltenango modifiers, and are semilethal with Willapa Bay modifiers (table 6). This chromosome is sensitive also to temperature and

population density variations. The fourth chromosome AA 1035 is normally viable with Mount Campbell modifiers, subnormal with Quezaltenango, Pachuca, and Willapa Bay, and poorly viable with Borego Valley modifiers. The chromosome PA 851 is normal with Borego Valley but subnormal with other modifiers.

No system or "geographic" modifiers is either favorable or unfavorable for all the San Jacinto chromosomes. The fact which deserves some emphasis is that the Borego Valley modifiers do not stand out as particularly favorable ones. Borego Valley is a locality close to San Jacinto, and one might have expected that homozygotes for the San Jacinto second and fourth chromosomes would display the highest viability in the Borego Valley genetic system. Yet, while Borego modifiers interact favorably with the chromosomes PA 851 and AA 1015, they are clearly deleterious for AA 1035. AA 1035 survives best with modifiers from Mount Campbell and Quezaltenango, which are geographically very remote.

The data were examined to detect whether or not the development rates and the sensitivities of the homozygotes for the San Jacinto second and fourth chromosomes to population density and other conditions in the cultures are subject to modification by the "geographic" genetic systems. The conclusion is that, as a rule, the development rates and the sensitivity to crowding and other culture conditions remain constant with all of the five geographic modifier systems tried. One exception to this rule, however, has appeared. In the experiments with constant modifiers, homozygotes for the second chromosome AA 1003 were found to develop very slowly; at 21° very few or no wild type flies appeared for two to three days after the beginning of the emergence of adults in the cultures, while toward the end of the hatching period mostly wild type and few or no Bare flies emerged. Yet, in every one of the ten cultures of AA 1003 with the Borego Valley modifiers, wild type flies were present in every count from the first on, and Bare flies did not disappear or become very rare till the end of the hatching period (table 7). In cultures with other modifiers, especially with those of the Pachuca strain, the hatching of wild type flies was long delayed (table 7). According to the artificial classification used by DOBZHANSKY, HOLZ, and SPASSKY (1942), the development rate of the AA 1003 homozygotes must be described as "slow" with Borego Valley modifiers, and as "very slow" with the other modifier systems tried.

As described above, the homozygotes for the fourth chromosome AA 955 have rough eyes and sometimes deformed legs, these changes being most pronounced at the temperature 25½°, less at 21°, and only rarely in the males but not in the females at 16°. All the experiments with the different modifier systems were carried, as stated, at 21°. Yet, with the Quezaltenango modifiers the homozygotes for AA 955 have extremely rough, frequently deformed, eyes, deformed legs, posterior crossveins frequently missing, wing margin cut or beaded. With the Pachuca, Willapa Bay, and Borego Valley modifiers the abnormalities are slight and confined mostly to males. With the Mount Campbell modifiers the homozygotes appear normal, at least at 21°. The changes in

TABLE 7

The development rate of homozygotes for the second chromosome AA 1003. Total numbers of the flies (n) and percentages of wild type among them (%) on different days of hatching in cultures with different "geographic" modifiers.

DAY OF HATCHING	MODIFIERS									
	BOREGO		QUEZALTENANGO		CAMPBELL		WILLAPA		PACHUCA	
	n	%	n	%	n	%	n	%	n	%
1	244	15.2	116	—	104	—	150	—	246	—
2	459	15.5	353	0.6	281	3.2	304	2.3	779	—
3	400	23.0	272	14.3	327	9.8	357	8.1	672	2.8
4	342	21.6	259	19.7	397	15.6	385	13.0	455	11.2
5	262	34.0	171	45.6	357	18.8	268	27.6	341	25.2
6	226	37.6	149	45.0	494	30.0	275	38.2	275	40.7
7	198	54.5	119	68.9	281	46.3	272	43.4	175	56.6
8	138	56.5	165	46.1	251	51.4	182	65.4	294	55.8
9	133	64.7	49	59.8	225	68.0	148	73.0	186	69.9
10	90	64.4	70	81.4	210	68.1	102	82.4	181	69.6
11	90	71.1	35	77.1	158	77.2	63	95.2	192	84.4
12	40	60.0	13	69.2	74	78.3	52	90.4	84	79.8
13	44	45.5	17	82.4	29	96.6	25	88.0	105	86.7
14	—	—	5	80.0	—	—	4	100.0	35	85.7
15	—	—	4	75.0	—	—	1	100.0	35	88.6
16	—	—	—	—	—	—	—	—	7	85.7

the manifestation of the "characters" just described may, therefore, be induced either by changing the temperature or by changing the genetic residues.

DISCUSSION

It is a well known fact that most mutants which appear in any species are deleterious to their carriers. This fact seems contradictory to the theory almost universally held by geneticists, that evolutionary changes result from summation of mutational steps. The contradiction is resolved by the following three supplementary hypotheses. First, since the adaptive value of a mutant is a function of the environment, a genotype which responds unfavorably in some may be neutral or useful in other environments. Second, since the adaptive value of a genotype is determined by gene interactions, a mutant gene which is unfavorable in combination with some genes may be neutral or favorable in other gene combinations. Third, the prevalent genotypes of a species being formed by many generations of natural selection in the environments in which the species normally lives, it is probable that any mutations, except the exceedingly rare ones, which are highly favorable in the normal environments have already been incorporated in the usual species genotype (the wild type).

The flaw in these supplementary hypotheses is that, although apparently reasonable, they rest on a rather meager observational basis. TIMOFEEFF-RESSOVSKY (1934) studied the viability of six sex-linked mutants in *Drosophila*

funebris relative to the viability of the wild type at three different temperatures and at different population densities. Some mutants are relatively more viable at low and others at high temperatures and population densities; the mutant eversae is superior to wild type at 24°–25° but inferior at 15°–16° and at 28°–30°C. Combining the mutants by two's, TIMOFEEFF-RESSOVSKY found that the viability of a compound may be lower than that of either mutant taken singly, equal to that of either mutant, intermediate between the two, or higher than that of either. KÜHN and HENKE (1932) found in the moth *Ephestia kühniella* that the gene *a*, which produces a deleterious effect on an otherwise wild type genetic background, has no such effect in combination with another mutant gene, *t*. Our results fall in line with those of the authors just cited. We have, however, studied the effects not of single mutant genes arisen in a laboratory but of chromosomes derived from natural populations. It is virtually certain that the different chromosomes in our material differ not in a single locus but in several and perhaps in many loci. We have studied not the effects of single genes but the net effects of combinations of genes located in the second and fourth chromosomes. Homozygosis for these chromosomes produces a great variety of genotypes. Some genotypes modify the viability, others the development rate, others the structural characters, the viability and the development rate, or the development rate and the structural characters of their carriers. Both negative and positive deviations from the "normal" viability and development rate are observed, the former, however, being more frequent and striking than the latter (*cf.* DOBZHANSKY, HOLZ, and SPASSKY 1942). The effects of some of the chromosomes on the viability are alike or nearly so at all the temperatures, population densities, and other culture conditions, and with all the genetic residues tried. Some genotypes respond by small and others by large modifications of their viability effects to variations in temperature, in population density, in genetic residues, or in combinations of these variables. No genotype has been found which would display a viability superior to normal in all external and genetic environments tried; some genotypes, however, do produce viabilities in excess of normal at certain special conditions. The modifications of the development rate seem to be on the whole less plastic in their response to environmental variations than are the viability modifications: a genotype which causes its carriers to develop slowly at one temperature will usually do so at other temperatures as well. One case, however, was found in which the retardation of the development is relatively greater with some than with other genetic residues.

The variety of genotypes with different reaction norms revealed in natural populations of *Drosophila pseudoobscura* is great indeed. Since we have examined the responses of genotypes homozygous for certain wild second and fourth chromosomes, the variety of reaction norms observed is, of course, not available at all times for natural selection to work with. This is true because in most natural populations so many chromosomes with different gene contents are known to be present that individuals homozygous for any one chromosome are much less frequent than heterozygotes. The variability of reaction norms

among heterozygotes is doubtless not so great as that among homozygotes. This fact permits setting up the somewhat artificial standards of viability, development rate, etc. "Normal" viability, "normal" development rate are averages displayed by individuals which have two second, two third, and two fourth chromosomes drawn at random from a given natural population; such individuals are mostly heterozygotes. Evidence is on record, however, that there is no complete uniformity in the reaction norms among individuals which compose at least some natural populations: genetic changes connected with the succession of the seasonal climatic cycles have been observed in populations of certain localities (DOBZHANSKY 1943). Some genotypes have selective advantages at certain seasons, balanced by disadvantages at other seasons.

We regard the diversity of reaction norms exhibited by homozygotes as a store of potential phenotypic variability. Owing to the recessivity of most of the component parts of this store, a major portion of the supply of genetic variability is carried in natural populations in a concealed condition, protected from natural selection. Nevertheless, the concealed and the expressed variabilities are interdependent, the latter originating, at least in part, through eruptions of the former on the phenotypic surface. Any recessive gene carried in a panmictic population in more than a single chromosome has a finite chance to become homozygous in any generation, this chance being greater in small than in large populations. The biological function of the concealed variability lies not only in that it conserves a store of genetic variants that are unfavorable in the existing environments but may be favorable in some environments in which the species will live in the future. The field of gene combinations which a species is capable of producing may include genotypes that are favorable but composed of elements unfavorable in other combinations. In terms of WRIGHT'S (1932) symbolism, a species may have several adaptive peaks separated by adaptive valleys potentially available to it, but some of the adaptive peaks may be difficult to reach unless the crossing of the intervening adaptive valleys is accomplished with natural selection temporarily relaxed or removed. The concealment of variability has a sheltering effect with respect to natural selection.

The gene contents of chromosomes are in a flux, unless suppressors of crossing over such as inversions are present. Since no inversions are present in the second and fourth chromosomes in the Mount San Jacinto populations, the particular gene combinations which we have studied are subject to frequent breakdowns and restorations in nature. The freedom of gene recombination is more limited in the third chromosome of *Drosophila pseudoobscura* because the same populations are in large part composed of inversion heterozygotes. Nevertheless, as far as the present data go, the concealed genetic variability is neither appreciably larger nor smaller in the third than in the second and fourth chromosomes (DOBZHANSKY, HOLZ, and SPASSKY 1942). The meaning of this difference between chromosomes of the same species remains obscure. An inversion which is in itself adaptively neutral would acquire a selective advantage, however, if it prevents the breakdown of a favorable gene combina-

tion formed in a given chromosome. It is known that third chromosomes with different gene arrangements are not adaptively equivalent in some cases (DOBZHANSKY 1943).

SUMMARY

The effects of 26 second and 22 fourth chromosomes of *Drosophila pseudoobscura* derived from populations of Mount San Jacinto, California, on the viability, development rate, and structural characters of homozygotes were studied. Each class of homozygotes was compared with sibs heterozygous for the same wild chromosome and for a chromosome provided with suitable genetic markers. The effects of these markers were examined in special control experiments. Cultures with the offspring of the same group of parents were raised at three temperatures (16½°, 21°, and 25½°C) and at different population densities. Some uncontrolled diversity of culture conditions was also present. The interactions of five second and four fourth chromosomes with genetic modifiers of each of the five wild strains from different parts of the distribution area of the species were investigated.

Homozygotes for some of the chromosomes show the same relative viability at all temperatures, population densities, and culture conditions tried. The viability of the other homozygotes was slightly, and of still others sharply, modified either by temperature, population density, culture conditions, or by combinations of these variables. In an extreme case, the same genotype is lethal at 25½°, semilethal at 21°, and almost normally viable at 16½°. In general, the viability optimum lies more frequently at the lowest than at the intermediate temperature, and no striking cases of optima at the highest temperature have been found. Some genotypes are more favorable at low, others at intermediate, and still others at high population densities. Viabilities superior to normal in some environments have been observed, but in no case has a genotype proved superior to normal in all the environments.

Relative development rate appears to be a more stable character than viability, since a chromosome which causes the homozygotes to develop slower than the controls in one environment usually does so in the other environments. One clear case, however, has been observed in which the retardation of the development is more pronounced with certain sets of genetic modifiers than with other sets.

Viability of every one of the nine homozygotes studied in this respect is influenced by genetic modifiers. However, the effects of the modifiers are more pronounced in some cases than in others. An extreme case was found of a variation from a slight deterioration of the viability to semilethality, with the temperature and culture conditions kept as constant as possible.

None of the five systems of geographic genetic modifiers proved to be uniformly favorable or unfavorable for all the homozygotes. Particularly, there is no obvious correlation between the geographic origin of a system of modifiers and its favorableness or otherwise. This is not unexpected. Since the homozygotes for any one chromosome are rare in wild populations, the geographic

modifier systems are not adjusted by natural selection to the properties of homozygotes.

One of the chromosomes produces a set of visible changes in the fly, which, with a standard modifier system, are extreme at 25½°, mild at 21°, and almost absent at 16½°. Altering the modifier systems, the same changes may be exaggerated or suppressed in flies raised at 21°. This is an excellent example of a parallelism of environmental and genetic changes.

Natural populations contain a tremendous variety of genotypes with different reaction norms.

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HETEROCARYOSIS IN NEUROSPORA CRASSA[1]

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IT IS possible by means of X-ray or ultraviolet treatment to produce mutant strains of *Neurospora crassa* that are deficient in their abilities to synthesize specific vitamins, amino acids, or other essential substances (BEADLE and TATUM 1941; TATUM and BEADLE 1942b). It occurred to us that it might be possible to determine whether the mutant genes concerned in such haploid strains are recessive or dominant to their normal alleles by producing heterocaryons, the cells of which would contain haploid nuclei from two mutant strains, one differing from wild type by one gene and the other differing by a second gene.

In our first attempt to produce a heterocaryon the mutant strains used were aminobenzoicless (unable to synthesize the vitamin *para*-aminobenzoic acid) and nicotinicless (unable to synthesize nicotinic acid). Growth rates were measured in special growth tubes (RYAN, BEADLE, and TATUM 1943) containing "minimal medium" (salts, sucrose, biotin, and agar, and containing neither *p*-aminobenzoic acid nor nicotinic acid). The two mutant strains were grown separately as controls. One experimental tube was inoculated with conidia of the two mutant strains of opposite mating types (sexes)—that is, the conidia were mixed at one end of the tube. It was supposed that the conidia would germinate and hyphae of opposite sex fuse. If both mutant genes were recessive, the two types of nuclei present in the fusion hyphae would complement each other, each type carrying the normal allele of the mutant gene in the other, and growth would occur. To determine the rate of any growth that might occur as a result of intercellular diffusion of complementary growth factors synthesized and excreted into the medium by separate hyphae of the two types, nicotinic acid by aminobenzoicless hyphae and *p*-aminobenzoic acid by nicotinicless hyphae, a tube was set up which was inoculated with conidia of the two mutants of the same mating type. Here no fusion was expected. However, the observed results immediately indicated that we were wrong in this expectation. The tubes in which like mating types were introduced gave growth rates similar to a wild type control, while growth was much slower in those in which different mating types were mixed. The control tubes in which single mutants were put showed little or no growth. Although we did not properly appreciate the fact at the time these experiments were carried

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out, hyphae which do not differ in sex reaction are known to fuse in many fungi (DE BARY 1887; WARD 1888; and many others), including species of Neurospora (KÖHLER 1929, 1930; SCHÖNEFELDT 1935; DODGE 1942). In many imperfect fungi such fusions can be shown, under experimental conditions, to lead to mixing of nuclei to give heterocaryotic strains (HANSEN and SMITH 1932; HANSEN 1938).

It was soon shown that unisexual heterocaryons can be produced at will in *Neurospora crassa* if the experimental conditions are so arranged that only heterocaryotic mycelia can grow. In this way the phenomenon of heterocaryosis provides a general method for determining dominance relations between mutant genes and their normal alleles. Furthermore, a method is made available by which tests for allelism in a haploid organism become as simple as in a diploid. It is the purpose of this paper to describe how heterocaryons are made, to offer proof that they really contain a mixture of two kinds of nuclei, to show how they can be used in studying dominance relations in a manner not possible in diploid organisms, and to point out some of the implications that have to do with the mechanisms of evolution in the fungi. The question as to whether mutant strains that are complementary in synthetic abilities could grow symbiotically if they did not fuse remains unanswered. Presumably they could, but since hyphae are actually found to be heterocaryotic, this condition is evidently more efficient than extracellular symbiosis.

PREVIOUS STUDIES

A remarkably clear demonstration of the occurrence of heterocaryosis in a fungus was made some years ago by HANSEN and SMITH (1932). These workers found that *Botrytis cinerea*, a species of the *Fungi Imperfecti*, contains an inconstant type called *x*. On culturing single multinucleate conidia of this type, it was found that some give rise to a constant type *a*, others give inconstant cultures, while still others give rise to a second constant type *b*. HANSEN and SMITH postulated that the *x* type is heterocaryotic, containing a mixture of two genetic types of nuclei. By chance assortment, some conidia contain only one type of nucleus, some only the other type, and some a mixture of both as expected on this assumption, type *a* strains give only type *a*, type *b* only type *b*, while type *x* gives *a*, *b*, and *x* types. This interpretation was shown to be correct by growing type *a* and *b* strains together. In this way type *x* was reconstituted through hyphal fusions and nuclear migrations. It should be emphasized that these phenomena, fusion of hyphae and random assortment of nuclei in the formation of conidia, are strictly asexual.

HANSEN (1938) has extended these observations to other species of the *Fungi Imperfecti*. In cultural studies of species from thirty genera, it was found that the available strains of more than half of these were heterocaryons. Single vegetative spore isolations gave rise to constant non-conidial forms, constant conidial forms, and inconstant mixed forms. The widespread occurrence of the phenomenon of heterocaryosis in certain groups of fungi is therefore

established beyond any reasonable doubt, although unfortunately the work of HANSEN and SMITH has received relatively little attention by investigators interested in this subject.

LINDEGREN (1934) reports that bisexual heterocaryons of *Neurospora crassa* may occur in nature and discusses the significance of these from the standpoint of the evolutionary economy of the species. He later (1942) discusses the possibility of unisexual heterocaryosis occurring in fungi either as a result of hyphal fusions or by mutation. It remained, however, for DODGE (1942) to produce unisexual heterocaryons experimentally in material that can be worked with genetically. By combining vegetatively the strains Dwarf 16 and C 8, both mating type *a* of *Neurospora tetrasperma* and both relatively slow growing types, a heterocaryotic strain was obtained that grew much faster and sporulated more abundantly than either component strain. Cultures derived from small conidia from such vigorous cultures gave rise to three types, C 8, Dwarf 16, and a vigorous type (the heterocaryon), thus indicating the presence within a single conidium of both C 8 and Dwarf 16 nuclei.

The work reported in this paper indicates that the method of DODGE may be extended to the heterothallic species *Neurospora crassa*.

MATERIAL AND METHODS

The significant feature of putting the phenomenon of heterocaryosis on a predictable experimental basis is the establishment of conditions which give the heterocaryon a strong selective advantage over the component strains. With mutant strains deficient in particular syntheses and hence dependent on an outside source of vitamins, amino acids, or other substances, such conditions are readily arranged by growing cultures on a medium deficient in the growth factors required by the two strains. If the mutant genes are both recessive to normal, the two kinds of nuclei complement each other in the heterocaryon so that only heterocaryotic hyphae can grow, and the heterocaryotic condition is automatically maintained. The same principle may be applied to strains that have low growth rates for other reasons—for example, colonial types that advance more slowly over an agar culture medium than do wild type strains. If one or both components are types with normal growth rates, but otherwise distinguishable from wild type, it is possible to make tests for dominance and allelism by first genetically combining such types with mutants unable to synthesize growth factors but morphologically normal in the presence of the growth factors required. Heterocaryotic combinations can then be detected by their ability to grow on a minimal medium. This can be illustrated with the hypothetical mutants *x* and *y*, each of which grows at a normal rate. By making the double mutants *x a* and *y b*, where *a* and *b* are biochemical mutants known to be recessive and non-allelic, the heterocaryon (*a*+*x*) plus (+*b y*) can be made. If this is morphologically normal, mutants *x* and *y* are recessive and non-allelic

In the experiments to be referred to, several mutants, all derived from wild

type strains of *Neurospora crassa* following treatment with X-rays or ultraviolet radiation, were used. Each of these behaves in crosses as though differentiated from the original wild type strain by one significant gene. Reference to mutants is made by numbers assigned at the time the original mutant spore was isolated. "Biochemical" mutants are named according to the substance they cannot synthesize, although it should be pointed out that in many instances it is not known that this is the immediate product of the blocked reaction. Thus a particular arginineless mutant might not be able to convert ornithine to citrulline (SRB and HOROWITZ unpublished) and therefore might more properly be called citrullineless.

The biochemical mutants made use of in studies reported here are as follows: Albino 1 (*al-1*)-4637, absence of pigmentation in mycelium and conidia.—albino 2 (*al-2*)-15300, phenotypically like albino-1 (MRS. M. V. G. HUNGATE unpublished).—aminobenzoicless (*pab*)-1633, unable to synthesize the vitamin *p*-aminobenzoic acid (TATUM and BEADLE 1942a).—arginineless (*arg*)-29997, unable to synthesize ornithine and hence citrulline and arginine (SRB and HOROWITZ unpublished).—lysineless (*lys*)-4545, unable to synthesize the amino acid lysine (DOERMANN unpublished).—nicotinicless-1 (*nic-1*)-3416, unable to synthesize the vitamin nicotinic acid (BEADLE, TATUM, HOROWITZ, and BONNER unpublished).—nicotinicless 2 (*nic-2*)-4540, unable to synthesize nicotinic acid. Genetically and biochemically different from nicotinicless-1 (BEADLE, TATUM, HOROWITZ, and BONNER unpublished).—pantothenicless (*pnt*)-5531, unable to synthesize pantothenic acid (BEADLE, TATUM, HOROWITZ, and BONNER unpublished).—tryptophaneless-1 (*tpt-1*)-10575, unable to synthesize the indole nucleus of the amino acid tryptophane (TATUM, BONNER, and BEADLE in press).—tryptophaneless-2 (*tpt-2*)-40008, unable to synthesize indole and hence tryptophane. Genetically and biochemically different from tryptophaneless-1 (TATUM, BONNER, and BEADLE in press).

In addition to these, four morphological mutants were used. These are referred to by number only. Brief descriptions follow: 221, a semi-colonial form that progresses along a growth tube at approximately one percent of the rate of a normal strain. Discolors medium somewhat.—2608, a colonial form that produces cauliflower-like buttons of growth. Rate of progression less than one percent of that of wild type.—3100, a colonial form like 2608. Turns dark and blackens the medium on which it grows.—5801, a semicolonial form, the mycelium of which gives the appearance of flowing over an agar surface. Rate of progression about one percent of that of wild type.

Mating types are designated by the letter *a* in upper or lower case. Thus 4637A is albino-1 of mating type or sex *A* which corresponds to the (+) type of LINDEGREN. Mating type *a* corresponds to the (−) sex of LINDEGREN.

Measurements of growth rates were made in special glass growth tubes as described in detail by RYAN, BEADLE, and TATUM 1943. In these the mycelium is allowed to progress along the surface of an agar medium. Measurements are made at 12-hour or other desired intervals. In general a minimal medium made up with Fries solution, sucrose (1.5 percent), biotin (0.005 μg/ml), and agar (3 percent) was used.

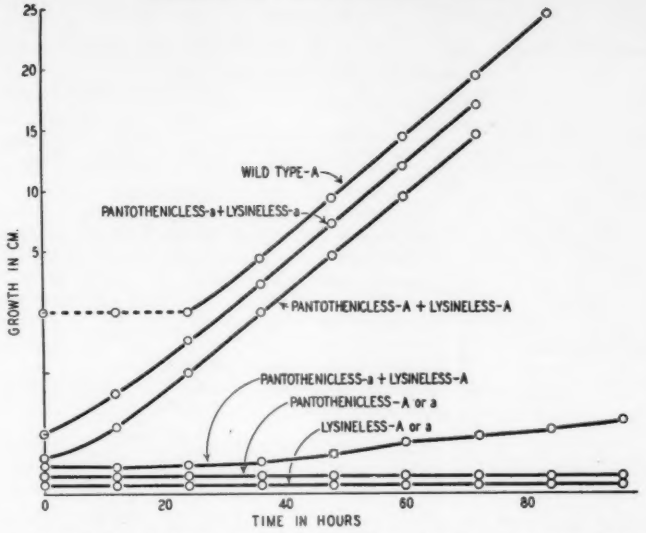

FIG. 1. Growth of lysineless, pantothenicless, wild type, and lysineless plus pantothenicless heterocaryotic strains of *Neurospora crassa*. For some reason the wild type culture did not start growing for 24 hours (dotted portion of growth curve), but this has no significance so far as the equilibrium rate is concerned.

GROWTH OF HETEROCARYONS

The behavior typical of heterocaryons made by combining two strains deficient in different growth factors is summarized in figure 1. The two components, pantothenicless and lysineless, show no measureable growth on minimal medium. When like-sexed strains of the two mutants are grown together on the same medium, however, the growth rate is as high as that of wild type—that is, about four millimeters per hour. When mutants of opposite sex are grown together, irregular growth occurs, averaging about 0.9 mm/hr.

Essentially similar results have been observed for other unisexual combinations, fourteen of which are listed in table 1. It is a general rule that bisexual heterocaryons show irregular growth as measured in tubes and have a lower average rate than do those that are unisexual. It is supposed that this lower growth rate of bisexual heterocaryons in *N. crassa* results from the observed tendency of hyphae containing nuclei of the two sexes to form fruiting bodies (perithecia) rather than to grow in the manner of strictly vegetative hyphae. The difference between the two types may well be mainly in orientation of growth rather than in its amount. In all combinations of group 1 and group 2 of table 1, like-sexed heterocaryons gave growth rates essentially the same as that of wild type controls—that is about 4 mm/hr. The combinations of two morphological mutants tested gave growth rates lower than normal. The two listed in table 1, group 3, gave rates of 2.5 and 2.0 mm/hr. These combinations will be discussed further in considering dominance relations.

TABLE 1

Combinations of mutants of the same sex that have been observed to give heterocaryons as judged by growth rates and phenotypic appearance.

STRAIN NUMBERS	NAMES OF MUTANTS	GROWTH RATES (mm/hr)
Group 1		
1633+ 3416	aminobenzoicless+nicotinicless-1	4.1
1633+ 4540	aminobenzoicless+nicotinicless-2	4.1–4.4
1633+ 5531	aminobenzoicless+pantothenicless	4.2–4.4
4540+ 5531	nicotinicless-2+pantothenicless	4.2–4.4
4545+ 5531	lysineless+pantothenicless	3.9–4.6
4545+10575	lysineless+tryptophaneless-1	4.1–4.3
4545+29997	lysineless+argininеless	4.4–4.5
5531+10575	pantothenicless+tryptophaneless-1	4.5
10575+29997	tryptophaneless-1+argininеless	4.2–4.4
Group 2		
221+ 5531	morphological+pantothenicless	4.2–4.4
2608+ 4545	morphological+lysineless	*ca.* normal
3100+ 5531	morphological+pantothenicless	4.2–4.4
5531+ 5801	pantothenicless+morphological	4.3–4.4
Group 3		
221+ 3100	morphological+morphological	2.2–2.6
221+ 5801	morphological+morphological	0.7–2.5

PROOF OF THE HETEROCARYOTIC CONDITION

In the case of five different heterocaryons, each made by combining two different mutant strains, bits of mycelia were removed from the frontier of the growing culture after these mycelia had migrated through growth tubes (about 30 cm). Such inocula were transferred to petri plates of minimal medium and allowed to develop for several hours at 25°C. During this time hyphae had grown out radially from the site of inoculation for a distance of several millimeters. Individual hyphal tips were cut off with a platinum-iridium microspatula and transferred to regular test tube slants of Difco cornmeal agar medium. Into these same tubes conidia of the wild type strain of unlike mating type were transferred. After fusion and production of ripe perithecia had taken place, samples of ascospores were removed and cultured individually on media fortified with the growth factors required by the components of the heterocaryon. If biochemical mutants were involved, such single ascospore cultures were transferred asexually (by means of conidia) to a minimal medium and to two sets of minimal media each fortified with a growth factor required by one of the components. This procedure as applied to the heterocaryon made by combining aminobenzoicless and nicotinicless strains is summarized as follows: (1) Aminobenzoicless and nicotinicless strains grown together.—(2) Combination culture grown through growth tube on minimal medium.—

TABLE 2

Recovery of mutant strains from crosses of wild type with single hyphal tip cultures derived from heterocaryons.

COMPONENT 1 OF HETEROCARYON	COMPONENT 2 OF HETEROCARYON	NUMBER OF HYPHAL TIPS ISOLATED	NUMBER GIVING BOTH MUTANT TYPES
1633a, aminobenzoicless	3416a, nicotinicless-1	10	7
1633A, aminobenzoicless	4540A, nicotinicless-2	5	5
1633A, aminobenzoicless	5531A, pantothenicless	5	2
4545A, lysineless	2608A, morphological	5	4
221a, morphological	5801a, morphological	6	6

(3) Bit of mycelium transferred to minimal medium in petri plate.—(4) Single hyphal tip cultures isolated and crossed with wild type of opposite mating type.—(5) Ascospores isolated and single spore cultures established on a medium containing both *p*-aminobenzoic acid and nicotinic acid.—(6) Asexual transfers made to a minimal medium containing neither *p*-aminobenzoic nor nicotinic acid. Cultures are thus classified as normal or mutant.—(7) Mutant cultures transferred asexually to two media, one containing minimal requirements plus *p*-aminobenzoic acid, the other the same except with nicotinic acid instead of *p*-aminobenzoic acid. Those that grow on *p*-aminobenzoic acid are aminobenzoicless; those that grow on nicotinic acid are nicotinicless.

If the mycelium is a true heterocaryon, at least some of the hyphal tips removed from it should contain both types of nuclei, and both mutants should be recoverable from the cross with wild type. Conversely, recovery of both component mutants constitutes proof of the heterocaryotic condition.

Results of this procedure applied to five heterocaryons are summarized in table 2. In all combinations both component mutants were recovered from at least some single hyphae. In the case of hyphae from which only wild type or only one type of mutant was recovered, the numbers of mutant cultures recovered were so small that sampling errors could reasonably account for the failure to recover both components.

These observations show that in the combinations studied our interpretation that growth is due to a true heterocaryotic condition is correct. There can be no reasonable doubt that it is a general truth for those pairs of slow or non-growing mutants that in combination show a normal or greatly increased growth rate.

DOMINANCE RELATIONS

If a heterocaryon made up of two mutant strains, each of which fails to grow at a normal rate, shows a normal growth rate, it seems justifiable to conclude that each of the two mutant types concerned is recessive to the alternative wild type condition. This is the situation with all the eleven

mutants listed in table 1. Each of them is a component of at least one heterocaryotic combination that shows normal growth.

It is found, however, that the two combinations involving two morphological mutants do not give normal rates. Yet each of the three mutants concerned is completely recessive in a combination with pantothenicless (group 2, table 1). To account for this seeming inconsistency, it is necessary to appreciate the fact that a Neurospora mycelium is made up of hyphal cells which are multi-

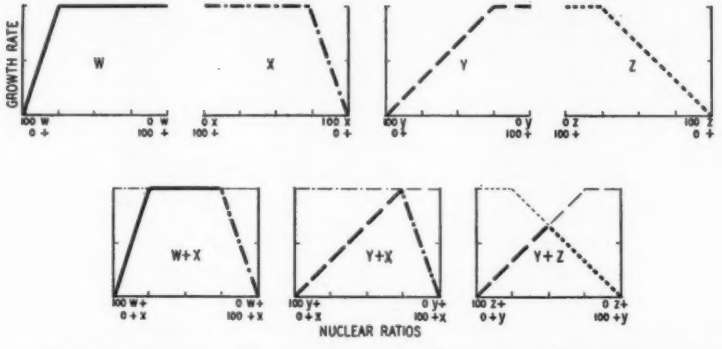

FIG. 2. Possible relations between nuclear ratios and growth rates of hypothetical heterocaryons.

nucleate and which are separated by crosswalls, each of which has a central perforation large enough to allow passage of nuclei from cell to cell. This means that in a heterocaryotic strain, in the absence of selection, there is no fixed ratio between the two (or more) types of nuclei present such as exists between chromosome sets in ordinary diploid or polyploid organisms. Instead, the ratio of the two kinds of nuclei in any "cell" of a two-component heterocaryon is free to shift continuously from nuclei all of one type to all of the other. If there is no selective advantage of hyphae with one nuclear ratio over those with another, the ratio in a given hypha will be governed by chance. However, in a heterocaryon made up of two components, each of which by itself has a sub-maximal rate of growth, there will be selection against hyphae with too great a proportion of either type. *A priori* there is no way of knowing within what range of ratios growth will be normal, but it should be possible to determine this experimentally. Some of the theoretically possible conditions for two-component heterocaryons are indicated in figure 2. Here four mutants *w*, *x*, *y*, and *z* are assumed to show the dominance relations represented in graphs W, X, Y, and Z. In these it is assumed that growth rates are linearly related to the proportion of wild type nuclei, but it should be emphasized that a linear relation is not necessary for the general interpretation. (See WRIGHT 1934, 1941, for discussion of physiological theories of dominance.) Strain *w* by itself—that is, with 100 percent *w* nuclei—does not grow. In heterocaryons with increasing proportions of wild type nuclei the growth rate increases until

at some ratio of the two kinds of nuclei a maximum growth rate is reached. This ratio of nuclei is assumed to be three mutant to one wild type in curve W. Any greater proportion of wild type nuclei would be as favorable. Here, then, a ratio of wild type to mutant nuclei of 1:3 or greater would be selected. Mutant *x* is assumed to show the same relation with wild type. Mutants *y* and *z*, on the other hand, are less recessive than *w* or *x* and therefore have less margin of safety. In heterocaryons with wild type they require that at least

TABLE 3

Summary of data on nuclear ratios in heterocaryons made by combining strains deficient in ability to synthesize specific growth factors.

MUTANT 1 MUTANT 2	*pab A* *pnt A*	*pab a* *pnt a*	*pab a* *pnt a*	*lys A* *pnt A*	*lys A* *pnt A*	*lys a* *pnt a*	*pnt A* *nic-2 A*	*pnt A* *nic-2 A*	*pab a* *nic-2 a*	*pab A* *nic-2 A*
Growth rate (mm/hr)	4.2	4.2	4.4	4.2	4.6	4.5	4.2	4.3	4.1	4.2
No. isolated	395	487	498	444	466	501	413	400	400	400
non-germinated	12	65	58	25	43	49	34	35	23	24
germinated	383	422	440	419	423	452	379	365	377	376
Wild type	201	217	218	213	231	242	251	186	227	239
Total mutants	182	205	222	206	192	210	128	179	150	137
Mutant 1	11	11	86	23	78	38	40	149	39	19
Mutant 2	171	194	136	183	114	172	88	30	111	118
Ratio $\frac{\text{mutant 1}}{\text{mutant 2}}$	$\frac{1}{15.5}$	$\frac{1}{17.6}$	$\frac{1}{1.6}$	$\frac{1}{8.0}$	$\frac{1}{1.5}$	$\frac{1}{4.5}$	$\frac{1}{2.2}$	$\frac{5.0}{1}$	$\frac{1}{2.9}$	$\frac{1}{6.2}$

three-quarters of the nuclei be wild type to give a normal growth rate. In all these cases, heterocaryons, in which wild type is one component, might by chance variation revert to wild type homocaryons. If, however, heterocaryons are made up of any two mutants showing the characteristics of *w*, *x*, *y*, or *z*, natural selection of rapidly growing hyphae will, under the appropriate conditions, automatically maintain the heterocaryotic condition. If mutants *w* and *x* are combined, the simplest prediction is indicated in curve W+X where it is seen the ratio of *w*+ to +*x* nuclei must remain between 3:1 and 1:3 for maximum growth. In other words, selection would be expected to maintain the ratio within these limits. In the same way it can be seen that if mutants *x* and *y* are combined (curve Y+X), the ratio would tend to be maintained at one *y*+ to three +*x* nuclei, the only ratio that gives maximal growth. Mutants *y* and *z* would give results as shown in curve Y+Z. Here no ratio gives a growth rate equal to wild type, but a maximum rate is given at a 1:1 ratio of the two types of nuclei.

On the basis of these considerations, the experimentally obtained growth rates for heterocaryons are understandable. Mutants 221 and 5801 each give normal rates with 5531 but with each other give a rate that is only about half that of a normal strain. Presumably pantothenicless (5531) is relatively more

recessive, like *w* and *x* of figure 2, while the morphological mutants 221 and 5801 are relatively less recessive to their normal alleles, like *y* and *z* of figure 2.

An indication that the nuclear ratios in a heterocaryon might deviate rather widely from a 1:1 was obtained in collecting the data of table 2. In testing for the recovery of the two components of heterocaryon 1633+5531 (aminobenzoicless+pantothenicless), of 33 mutant strains recovered, only two were aminobenzoicless. This indicates a difference in relative dominance of the normal alleles of the two mutant genes.

More extensive studies on the nuclear ratios characteristic of various heterocaryons are summarized in table 3. The method used in obtaining these results was as follows: Heterocaryons were allowed to grow through growth tubes and conidia permitted to form at the end opposite the site of inoculation. These conidia were then dusted onto wild type protoperithecia of the opposite mating type grown on cornmeal agar. Perithecia were allowed to ripen and discharge their ascospores. From tubes in which at least several hundred perithecia had developed, a random sample of 400 to 500 discharged ascospores was removed and the spores cultured individually. Half of these were expected to give rise to wild type cultures and the other half to mutants of one or the other of the two types combined to give the heterocaryon. These were then differentiated by growing asexual transfer cultures on media containing known supplements as described on page 296.

There are several possible sources of error. It is assumed that the nuclear ratio does not change during the process of formation of conidia. While this appears to be an entirely reasonable assumption, there is no proof that it is correct. Differential germination of ascospores of the two mutant types will give rise to spurious ratios. A check on this is had in the observed ratio of wild type of mutant cultures. If this is one-to-one as expected, it is a reasonable deduction that spore germination was satisfactory. This test was fairly satisfactorily met by all combinations in table 3 except those involving nicotinicless-2. This mutant is known from independent evidence to show reduced ascospore viability. In this respect, this particular mutant was an unwise choice.

It is seen that the ratios vary rather widely for different combinations and also in different determinations within one combination. This would suggest that the situation in these particular combinations is similar to curve W+X of figure 2—that is, in each there is a rather wide range of ratios that give wild type growth rates.

In the eight determinations in which pantothenicless was one component of the combination, the estimated percentage of pantothenicless nuclei varied from 31.2 to 94.6 percent. This means that the mutant gene concerned is relatively recessive. In the instance in which the percentage of pantothenicless nuclei was 94.6, one normal allele per seventeen was apparently sufficient to give a normal growth rate.

Since heterocaryons combining 3100 with 221 give low growth rates, it is probable that a rather high proportion of normal alleles is necessary to "cover"

each of these two mutants. An attempt was made to determine the ratio of 3100 to 5531 nuclei in a heterocaryon made up of these two mutants. The results were unsatisfactory because of poor viability of 3100, but they did suggest a marked preponderance of pantothenicless over 3100 nuclei, possibly as much as 50:1. This is consistent with the assumption that the allele of

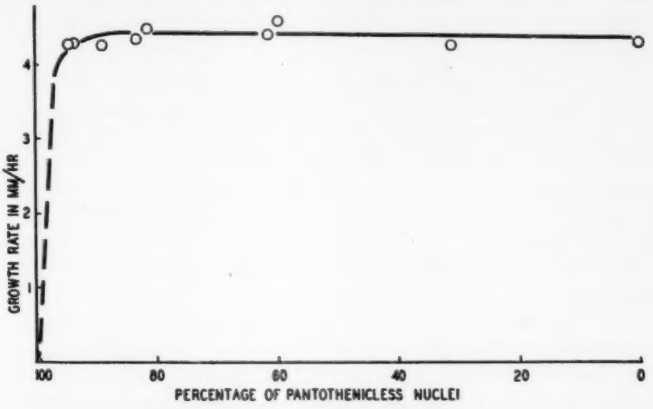

FIG. 3. Observed growth rates of heterocaryons in which pantothenicless was one component and in which the indicated percentages of pantothenicless nuclei were found.

pantothenicless is strongly dominant over its mutant allele, but that the normal allele of 3100 is only weakly dominant over the 3100 mutant allele.

Heterocaryons that do not grow at normal rates should have a more or less constant nuclear ratio if the theoretical considerations presented here are sound. Unfortunately, data that bear directly on this are not available.

If the general interpretation expressed in this paper is correct, it should be possible in heterocaryons to study dominance relations quantitatively and in detail. Given growth rate data and nuclear ratios on a sufficient number of combinations one should be able to draw complete growth rate-dosage curves for the mutants concerned. On the basis of the data presented it is already possible to define to a certain extent the relation between growth rate and the ratio of mutant to normal alleles of the pantothenicless gene. This is done in figure 3, where growth rates at experimentally determined ratios are recorded.

USE OF HETEROCARYONS IN TESTS OF ALLELISM

Three examples will serve to illustrate the use of heterocaryons in tests for allelism. Nicotinicless-1 and nicotinicless-2 are both sex-linked but appear to be biochemically different in that under certain conditions nicotinicless-2 produces a yellow pigment which is excreted into the medium, whereas nicotinicless-1 does not. It was found that when a mixed inoculum of these two mutants is put on a minimal medium, growth occurs which, when measured in

growth tubes, is approximately normal in rate. This strongly suggests that the component nuclei are *nic*-1+ and +*nic*-2. Direct confirmation of this was had by crossing the two mutants. Of 89 cultures produced from the resulting ascospores, 34 were wild type. With independent assortment of the two gene pairs, 25 percent wild type would be expected, with linkage, less than this. The observed excess is consistent with independent evidence that nicotinicless ascospores of both types show a lower germination percentage than do wild type ascospores.

The two tryptophaneless mutants, 10575 and 40008, show a similar relation. When like sexes are put together on minimal medium, growth is markedly accelerated over that of the single mutants, each of which grows slowly under these conditions. That they are genetically different is shown by the fact that from crosses between them, wild type strains are recovered. They are biochemically different as shown by the fact that 40008 will grow on anthranilic acid (*ortho*-aminobenzoic acid) while 10575 fails to do so (TATUM, BONNER, and BEADLE, in press). The evidence is clear that different genes have mutated to give rise to these two tryptophaneless strains.

A third example comes from the work of MRS. M. V. G. HUNGATE who kindly permits it to be cited prior to publication. It is observed that when albino-1 and albino-2 strains are grown together on a regular yeast extract-malt extract-agar medium, yellow pigment is produced in the conidia. These mutants are both sex-linked. From crosses between them, wild type strains are recovered with a low frequency indicating close linkage. However, there is genetic evidence suggesting that albino-1 is associated with a chromosome aberration of some sort so that the two genes may not be as close together as the data might indicate. Here again two phenotypically similar mutants appear to be differentiated from wild type by different genes. In this case mutual heterocaryotic dominance is expressed in pigment production rather than by an increased growth rate.

Other examples could be cited in which mutant genes of independent origins are indicated by heterocaryon tests to be allelic. A negative result, however, must be interpreted with caution. The mutant types may each require such a high ratio of normal alleles to give good growth that the required ratios are impossible to attain in the heterocaryon between them (an extreme case of the kind of relation illustrated in figure 2, curve Y+Z). This is equivalent to saying that one or both of the two genes is dominant in the ordinary sense in which this term is used in diploid organisms.

Although there is no fusion of nuclei, dominance relations presumably have the same physiological basis in heterocaryons as in ordinary diploid or polyploid organisms. For many characters, at least, it does not matter that homologous chromosomes carrying the two alleles of a given gene are separated by nuclear membranes. This relation has been shown to hold for many fungi as indicated by numerous reports in the literature, for a few special cases in higher plants, and for at least one instance in animals (see BULLER 1941 and POWERS 1943 for discussion and references). In the latter case it is shown that

individuals of *Euplotes patella* with two sets of diploid nuclei, each homozygous for mating types genes, show the same mating type as do animals with a single set of diploid nuclei heterozygous for the same mating type alleles (POWERS 1943).

USE OF HETEROCARYONS IN CROSSING MUTANT STRAINS

It is often observed that mutant strains of Neurospora form perithecia poorly and hence are difficult to cross. For example, the tryptophaneless strains 10575 and 40008 do not cross readily with each other. On the other hand, the unisexual heterocaryon in which both 10575 and 40008 nuclei are present is essentially wild type in behavior. It forms protoperithecia abundantly and can be crossed with the opposite sex of either tryptophaneless component. From such crosses, two types of asci are, of course, expected: those which segregate for both tryptophaneless gene pairs and hence give spores of wild type constitution, and those from ascospore mother nuclei homozygous for the tryptophaneless strain to which the heterocaryon was crossed.

This method, which can be used generally, is analogous to using heterozygotes in crosses of diploid organisms.

POSSIBILITY OF HETEROCARYONS WITH MORE THAN TWO COMPONENTS

It should be possible experimentally to produce heterocaryons with three kinds of nuclei all of which must be present to maintain normal growth. If three non-allelic recessive mutants *a*, *b*, and *c*, each of which fails to grow or grows slowly under the conditions selected, are combined in pairs to give the three strains $ab+$, $a+c$, and $+bc$, there should be formed, on growing the three double mutants together, a heterocaryon dependent for its growth on one normal allele in each of the three component nuclei. Natural selection would maintain the system as long as growth remained an advantage.

In a similar way with four mutant strains, four-component heterocaryons should be possible. Heterocaryons with more than two components might well be useful in studies on dominance relations.

SIGNIFICANCE OF HETEROCARYOSIS IN RELATION TO HETEROSIS

It is generally accepted that heterosis in diploid organisms is the result, at least in part, of dominant or semi-dominant favorable growth genes in one of the constituent chromosome sets complementing those of the other set (JONES 1942, see also EAST 1936). ROBBINS (1941) has suggested the possibility that increased vigor in excised hybrid tomato roots as compared with those of the parent strains may be the result of increased ability of the hybrid through complementary gene action to produce a full quota of growth factors and has suggested to DODGE (1942) that a similar interpretation might be applied to the cases of rapidly growing heterocaryons reported in *Neurospora tetrasperma*.

This interpretation applies to the observations presented here—for example, in the case of a heterocaryon made up of pantothenicless and lysineless (fig. 1) it is clear that ability to grow is the result of the complementary action of the

normal alleles of the two mutant genes concerned. Pantothenicless nuclei carry normal alleles of the lysineless gene, whereas the nuclei containing lysineless carry normal alleles of pantothenicless. The heterocaryon is thereby enabled to synthesize both pantothenic acid and lysine. Here, then, is what amounts to an extreme case of heterosis, clearly resulting from the presence of genetically complementary nuclei in a common cytoplasm. It is an instance in which it can be stated with confidence that the complementary growth factors are to be referred to the complementary genes concerned with the production of the vitamin pantothenic acid and the amino acid lysine.

The physiological basis of this cooperative gene action is with little doubt similar to that for hybrid vigor, or heterosis, in diploid organisms (DODGE 1942).

EVOLUTIONARY SIGNIFICANCE OF HETEROCARYOSIS

Accepting the text-book accounts of its life history, it is difficult to imagine how a haploid heterothallic fungus like *Neurospora crassa* could possibly compete in an evolutionary sense with a homothallic relative like *N. tetrasperma* in which two sets of chromosomes in separate nuclei are maintained in the mycelium. In *tetrasperma*, mutant genes which are deleterious in the combination in which they happen to first arise but potentially useful in other combinations can be saved through protection by their normal alleles. In *crassa* the opportunities for "trying out" new combinations would be greatly restricted if all mycelia were homocaryotic.

LINDEGREN (1942) has clearly pointed out that the phenomenon of heterocaryosis provides a mechanism by which this difficulty can be avoided. As a matter of fact, heterocaryons, with the possibility of several components and with no restrictions on the proportions of different chromosome sets present are even more flexible than diploids. Their formation in nature is inevitable. Hyphae of a single mycelium can be seen to fuse and anastomose freely. The same is true of hyphae from different mycelia. Dominance relations would be expected to give rise to "heterocaryotic vigor" following such interstrain fusions, and this vigor is all that is needed to maintain the heterocaryotic condition through natural selection.

That strains with normal growth rates, and with no apparent differences from wild type strains except those of pigmentation, do actually fuse and produce heterocaryons when grown together on a medium on which both components are able to grow is demonstrated by the behavior of albino-1 and albino-2 mutants as observed by MRS. M. V. G. HUNGATE. Here the selective advantage of the heterocaryon is not apparent, but, judging by pigmentation, the heterocaryotic condition invariably arises when the two strains are grown together on a yeast extract-malt extract-agar medium.

POSSIBLE RELATION OF HETEROCARYOSIS TO EVOLUTION OF SEXUAL REPRODUCTION

By greatly increasing the rate of formation of new combinations of genes, sexual reproduction has an obvious selective advantage over asexual means

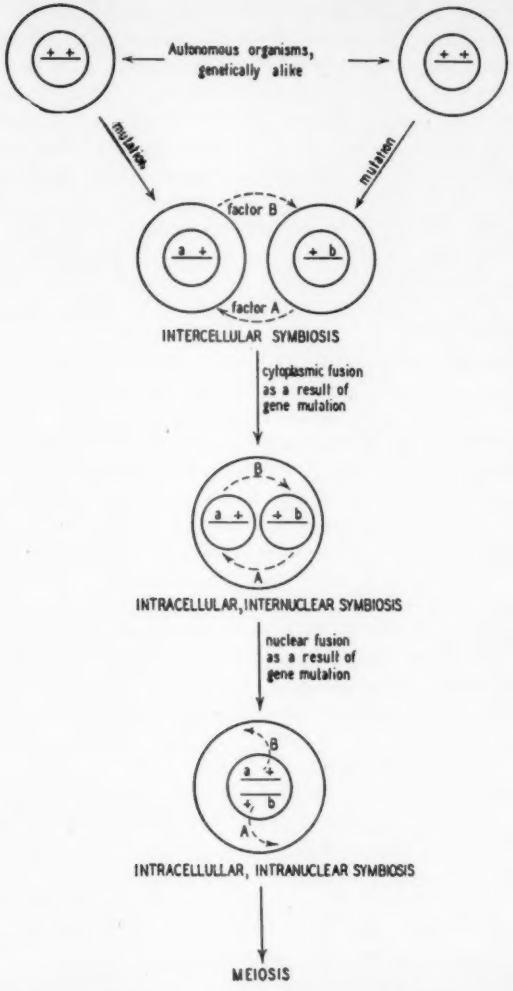

FIG. 4. Schematic representation of postulated steps in the evolution of sexual reproduction.

of multiplying individuals of a species. In considering possibilities as to how mechanisms of sexual reproduction evolved, DARLINGTON (1939) points out that nuclear fusion and meiosis must have arisen simultaneously since one without the other would be not only useless but indeed detrimental. He suggests that meiosis is a consequence of nuclear fusion and that the ability of a diploid nucleus to divide mitotically is a derived condition. In this way the

two events, fusion and meiosis, could arise as a result of a single evolutionary change. There still remains, however, the question of how cellular and nuclear fusion evolved. It seems possible that heterocaryosis, as it occurs in Neurospora, might have been one of the steps in this process. By this we do not mean to imply that sexual reproduction arose first in the fungi or that it arose only once. The hypothesis suggested could apply in principle to any of a number of primitive organisms. For example, myxomycetes, many algae, and numerous protozoa, particularly those with multinucleate cells, would appear to offer favorable opportunities for the phenomenon of heterocaryosis.

The scheme presented here proposes a series of successive steps, each of which, under appropriate circumstances, would have a selective advantage over the preceding condition. These conditions are represented in figure 4 and outlined as follows:

1. Organisms homogeneous and relatively autonomous in growth factor synthesis.

2. Differentiation of individuals through gene mutation into two groups deficient in abilities to synthesize growth factors. This could occur under environmental conditions such that these growth factors were not necessary for survival—that is, under conditions in which they were available from an external source. In fact, it is possible that it may be more economical than not for an organism to lose the ability to synthesize a substance that it can obtain in sufficient amounts from an external source.

3. Intercellular symbiosis arising from a change in environment which would reduce the availability of externally supplied growth factors, thus making survival of two deficient types dependent on the symbiotic relation between them. Such symbiotic pairs of organisms have been set up experimentally where the complementary forms are different species (*cf.* SCHOPFER 1943 for discussion and references). There is no apparent reason why strains of a single species should not cooperate in a similar way.

4. Vegetative fusion of cells without nuclear fusion with an increase in the efficiency of the symbiotic relation. This condition represents a stable condition that could persist more or less indefinitely, but one that is reproductively inferior to the succeeding stage.

5. Nuclear fusion with a still further increase in the efficiency of the complementary gene action. This step would presumably lead to an unstable condition at the next nuclear division such that chromosome pairing would occur, meiosis rather than mitosis would result, and the cycle could then be repeated.

It is seen that each step in the process outlined reduces the size of the combining units through the series: cell, nucleus, chromosome, and gene. Each advance would therefore increase the evolutionary flexibility of the species.

SUMMARY

Mutant strains of *Neurospora crassa* of the same mating type undergo vegetative fusion to form heterocaryons—that is, mycelia containing a mix-

ture of two types of nuclei. If each mutant involved is recessive, the heterocaryon may be phenotypically normal. If the two mutant components are deficient in their abilities to synthesize specific growth factors, such as vitamins or amino acids, the heterocaryon will be maintained by natural selection if it is grown on a medium that does not supply the growth factors necessary for growth of the individual components.

By crossing a strain derived from a single hyphal tip of a heterocaryotic mycelium to wild type, both component mutant strains used to make the heterocaryon can be recovered. This proves that the single hypha actually contained nuclei of both mutant types.

The ratio of nuclei in a heterocaryon is not mechanically fixed. If selection of rapidly growing hyphae is allowed to go on for some time, one would expect nuclear ratios to adjust themselves so as to give a maximum rate of growth. Such ratios have been measured in a number of heterocaryotic combinations and certain deductions made as to the dominance relations of the genes involved.

Heterocaryons can be used as tests for allelism and are often useful in crossing mutant strains that are otherwise difficult to hybridize.

It is theoretically possible to make heterocaryons with three or more components that will be maintained by natural selection.

Heterocaryotic vigor is believed to be similar in its physiological basis to heterosis in diploid or polyploid organisms.

The phenomenon of heterocaryosis serves to give the haploid mycelia of the heterothallic species of Neurospora at least some of the advantages enjoyed by diploid organisms in evolutionary competition.

It is suggested that a heterocaryotic condition might have been one of the steps in the evolution of sexual reproduction.

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